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THE ELECTRIC CHARGE OF AMINO ACID CRYSTALS IN AQUEOUS ELECTROLYTES

BY HAROLD A. ABRAMSON

(From the Department of Biological Chemistry, College of Physicians and Surgeons, Columbia University, New York)

The isoelectric points of microscopic crystals of l-cystine, of l-tyrosine, and of l-aspartic acid in HCl are between pH 2.3 and 2.5. The crystals are negatively charged at the isoelectric points (as calculated from the mass law) of the dissolved amino acids. In each instance reversal of sign of the crystal charge occurs at a pH close to the increase of the solubility of the amino acid. The curves relating the electric mobility of the amino acid crystals with pH resemble those of an inert substance like n-propyl benzene except that the latter is not reversed in sign of charge by acid. The data give some information in regard to the orientation of polar groups at the limits of the crystal lattice of ampholytes.

METABOLISM STUDIES FOLLOWING THE ADMINISTRATION OF GLYCINE

BY MILDRED ADAMS

(From the Section of Clinical Metabolism, The Mayo Clinic, Rochester, Minnesota)

The changes produced in the urine by feeding glycine alone or glycine and ephedrine appear to vary considerably, and to obtain further information concerning these, data have been obtained on normals, patients with myasthenia gravis, and patients with muscular dystrophy. The changes in the various nitrogen and sulfur partition products and phosphate were studied.

The increase in creatine, which seems to be an important factor in muscular metabolism, appears to be influenced by the degree of the creatinuria before glycine is administered. The increase in uric acid excretion is also variable. The results of a balanced experiment on one patient with myasthenia gravis show no decided change in the nitrogen, phosphorus, or sulfur balance, by feeding glycine.
THE PHYSIOLOGICAL PROPERTIES OF THE THYROTROPIC HORMONE

By E. M. ANDERSON

(From the Department of Biochemistry, McGill University, Montreal, Canada)

With a highly purified extract of the anterior pituitary containing the thyrotropic hormone, hyperplasia of the thyroid with hyperthyroidism has been produced in the rat and guinea pig; also complete replacement therapy with respect to the thyroid has been obtained in the hypophysectomized rat.

IDENTIFICATION OF TREHALOSE AS THE HIGHER ALCOHOL COMBINED IN THE ACETONE-SOLUBLE FAT OF THE TUBERCLE BACILLUS

By R. J. ANDERSON and MELVIN S. NEWMAN

(From the Department of Chemistry, Yale University, New Haven)

In previous investigations dealing with the composition of the acetone-soluble fat obtained from the tubercle bacillus, Strain H-37, it has been found that the fat contained at most mere traces of glycerol, and it was assumed that some higher polyhydric alcohol or a carbohydrate was present. It has now been shown that the higher alcohol in question is trehalose.

It is an interesting fact that the principal lipid fractions of the tubercle bacillus contain entirely different polysaccharides. The polysaccharide contained in the phosphatide yields on hydrolysis three sugars; namely, inositol, mannose, and some other reducing hexose sugar. The wax fraction contains a carbohydrate complex which consists principally of d-arabinose, galactose, and mannose. The acetone-soluble fat contains the crystalline disaccharide trehalose, C_{12}H_{22}O_{11} + 2H_{2}O.

THE OXIDATION OF CYSTINE IN ACID SOLUTION

By JAMES C. ANDREWS

(From the Department of Physiological Chemistry, School of Medicine, University of Pennsylvania, Philadelphia)

Further studies have been made of the mechanism of cystine oxidation in various acid solutions by pure oxygen. Solutions of
Z-cystine in HCl, H₂SO₄, and H₃PO₄ have been used in conjunction with various catalysts and the rate of uptake of oxygen has been measured. Temperatures of both 38° and 60° were employed and the rate both of racemization and oxidation was measured.

The great effectiveness of copper as an oxidative catalyst is emphasized by these studies. Although copper has been shown to accelerate the oxidation of sulfhydryl compounds to disulfides, its catalytic value in accelerating the further oxidation of a disulfide to the sulfonic acid has not previously been demonstrated. The amounts required are in excess of those normally present in an average good sample of cystine, several times recrystallized. Hence no attempt has been made to work with absolutely copper-free preparations. For example, at 60° 3.00 gm. of cystine in 600 cc. of 5 N HCl took up no measurable amount of oxygen in 1 week. Introduction into the sample of 10⁻⁴ mols of Cu⁺⁺ started immediate absorption of oxygen at the rate of about 20 cc. per day until the theoretical amount required for cysteic acid formation was absorbed. Analysis of the resulting solution showed inorganic sulfate corresponding to about 25 per cent of the original cystine, and the remainder of the sulfur in the form of cysteic acid, which was isolated and identified in the usual way. Determinations by the Folin method showed the complete absence of cystine. Iron was shown to be entirely ineffective as a catalyst in similar experiments. In this case, determinations by the Folin method showed a recovery of 94 per cent of the cystine, after the removal of which a small amount of cysteic acid was identified. This result corresponds to that obtained with no added catalyst.

A series of similar comparisons at 38° gave the same results. Copper showed very marked catalytic activity in amounts of 10⁻⁴ and 10⁻³ mols. Fe⁺⁺ and Fe⁺⁺⁺ in similar quantities both proved ineffective in 5 N HCl. In 5 N H₂SO₄ and 5 M H₃PO₄ 10⁻⁴ mols of Cu⁺⁺ were without effect. The combination of Fe⁺⁺ with Cu⁺⁺ in 5 N HCl did not increase the rate of oxygen absorption over that of the copper alone.

The formation of inorganic sulfate in these oxidations led to some experiments to determine whether it could be the result of further oxidation of the cysteic acid (for example, to serine or pyruvic acid and H₂SO₄). However, repeated attempts to effect such an oxidation of pure cysteic acid, both with O₂ and with Br₂, have thus
far given uniformly negative results and no catalyst as yet tried
has shown any effectiveness in promoting such a reaction. Fur-
thermore, all attempts to isolate serine or pyruvic acid from cystine
oxidations, in which inorganic sulfate was formed, have given
negative results.

THE PREPARATION AND PROPERTIES OF CYSTEIC ACID PHENYL
HYDANTOIN

BY JAMES C. ANDREWS AND KATHLEEN CRANDALL ANDREWS

(From the Department of Physiological Chemistry, School of Medicine,
University of Pennsylvania, Philadelphia)

In order to utilize the greater reactivity of cyclic amino acid
derivatives to accomplish, if possible, the preparation of taurine
from cysteic acid, the phenylhydantoin of cysteic acid has been
prepared. All attempts to form the hydantoin directly from
cysteic acid have given negative results but oxidation by means of
bromine of l-cystine phenylhydantoin gives the desired compound
in the form of its hydrobromide.

Cysteic acid phenylhydantoin hydrobromide crystallizes in
flat fragile plates which are quite water-insoluble but moderately
soluble in 95 per cent alcohol and very soluble in acetone.

Results of analyses for N, S, and Br correspond closely to the
theoretical. The Br can be titrated directly by means of silver
nitrate and is thus not substituted in the benzene ring. The sul-
fonic group and the hydrobromic acid may be titrated at very low
pH (about pH 3). At higher pH values the hydantoin ring splits
between positions (3) and (4), thus forming the hydantoic acid and
liberating a carboxyl group which is completely titrated by stand-
ard alkali at a methyl red end-point. Neither the hydantoin nor
the hydantoic acid gives any free amino nitrogen in the Van Slyke
reaction.

In the presence of excess bromine the compound is unstable at
room temperature, being slowly oxidized with apparently complete
destruction of the hydantoin ring and evolution of free nitrogen.
The absorption of certain sulfur compounds from intestinal loops of dogs

By James C. Andrews and Charles G. Johnston

(From the Department of Physiological Chemistry and the Department of Research Surgery, School of Medicine, University of Pennsylvania, Philadelphia)

As a preliminary to some metabolic studies of certain sulfur compounds, the relative rates of absorption of these compounds from jejunal loops of dogs were studied. The loops were prepared by the technique described by Johnston which permits keeping the animals in a healthy condition for many months. Weighed samples of the substances studied were introduced quantitatively into the loop and removed at the end of the experimental period (usually 4 hours). To insure quantitative recoveries the loops were washed repeatedly. Preliminary tests showed that it was possible to recover from 95 to 100 per cent of the material when removed immediately after its introduction.

The rate of absorption of the following compounds was measured: L-cystine, dl-cystine, cysteine, cysteic acid, and sodium sulfate. 0.5 gm. samples of cystine were used and in the case of other compounds equivalent amounts in terms of sulfur. The results showed some variation among different animals, but were very consistent for any one animal. One dog (18.3 kilos) gave the following recoveries on 4 hour experiments (maximum variation): L-cystine, 68 to 78 per cent; dl-cystine, 58 to 76 per cent; cysteine, 15 to 46 per cent; cysteic acid, 0 per cent; sodium sulfate, 68 to 82 per cent. In 2 hour periods the recoveries of cysteic acid for this dog varied from 16 to 24 per cent. With a larger dog (27.9 kilos) similar relationships were observed, but more rapid absorption. Individual differences among the animals were much more apparent with the use of the amino compounds than with sodium sulfate.

Simultaneous introduction of both cystine and cysteic acid gave recoveries identical with those obtained when either was introduced alone.

After administration of cystine, nitroprusside tests were always made on the recovered material but were uniformly negative. After administration of cysteine, nitroprusside tests were usually

negative, indicating that considerable reoxidation had taken place. This implies a higher rate of absorption for pure cysteine than is apparent from the above figures.

The slightly increased speed of absorption of $dl$-cystine over $l$-cystine confirms the results reported by Lawrie with rats. The difference, however, as was the case with Lawrie's experiments, is not very significant.

Variations in the volume of water introduced with the sample had no detectable effect nor did preliminary fasting of the animal influence the rate of absorption.

CHANGES IN TEETH OF RATS CONSUMING A RATION EXTREMELY LOW IN INORGANIC SALTS*

By S. S. ARNIM, MIRIAM F. CLARKE, B. G. ANDERSON, and ARTHUR H. SMITH

(From the Departments of Pathology and Physiological Chemistry, Yale University, New Haven)

The animals (albino rats) used in the present study grew at a rapid rate after weaning to a body weight of 100 gm. at approximately 35 days of age. At this time the experimental group was given a ration composed of purified foodstuffs extremely poor in mineral salts but otherwise adequate. The approximate daily intake of total salts by the rats on the low salt ration was 56 mg., the phosphorus 16 mg., and the calcium less than 1 mg. This ration was continued for 12 weeks, at which time some of the animals were killed ("12 + 0" in Table I) and others realimented for 4, 8, or 12 weeks ("12 + 4," "12 + 8," "12 + 12" in Table I) with a similar diet containing an adequate salt mixture. The age control rats received the adequate synthetic food ad libitum throughout and the calorie controls received this diet in the same amount as was consumed by the animals on the low salt ration. Controls were killed at the same time as were the experimental rats. Serial sections 5 to 8μ thick were made from one side of the lower jaw or from one side of both upper and lower jaws.

Lesions were found in the cusps and in the sulci of the molar teeth of some of these rats. No attempt was made to record the total

* Presented before the American Society for Experimental Pathology.
number of lesions found in one animal, but it was recorded as
carious if one or more lesions were found. The results indicate
a possible difference between some of the etiological factors of the
cusp lesion and those of the sulci lesion.

The cusp lesions are associated with tracts of dentin that were
in the process of formation during the period of salt deprivation.
These tracts of dentin are in that portion of the cusp which was
occupied by the pulp at an earlier stage of dentin formation.
The rats consuming a diet extremely low in inorganic salts deposit
tracts of very irregular dentin which contain connective tissue
cells and many spaces and appear to be poorly calcified. These
tracts of dentin extending into the cusps are points of structural

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>12 + 0</th>
<th>12 + 4</th>
<th>12 + 8</th>
<th>12 + 12</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet group</td>
<td>Low salt</td>
<td>Age control</td>
<td>Calories control</td>
<td>Low salt</td>
<td>Age control</td>
</tr>
<tr>
<td>No. of animals</td>
<td>16</td>
<td>2</td>
<td>4</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Caries Cusp</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Caries Sulci</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

weakness. The cusps wear off as the animals grow older until the
irregular tracts of dentin are near the occlusal surface. Frac-
tures may occur through this dentin, and necrosis of the pulp and
rat caries may follow.

The sulci lesions are not associated with portions of the teeth
which were forming during the period of salt deprivation. The
lesions are found involving enamel formed before the rats were
given the low salt diet. There is a wide spread of the process at
the dentino-enamel junction and an invasion of the dentin. This
study throws little light on the etiology of this type of lesion.
A TECHNIQUE FOR THE SPECTROPHOTOMETRIC STUDY OF UNDILUTED BLOOD

BY J. HAROLD AUSTIN AND DAVID L. DRABKIN

(From the John Herr Musser Department of Research Medicine and the Department of Physiological Chemistry, School of Medicine, University of Pennsylvania, Philadelphia)

In the course of some of our spectrophotometric studies a technique in which undiluted blood could be used appeared necessary. We have designed accordingly a cell which permits the study of defibrinated or oxalated blood as drawn and without exposure to air or alteration by diluents not in gaseous equilibrium with the blood.

The chamber of the cell, ground out of a plate of optical glass, is 0.07 mm. in depth, and is readily filled and emptied by means of two glass capillary tubes, connected with the chamber by ground openings in a heavy plate glass cover, forming the floor of the chamber.

When saponized blood is used, oxyhemoglobin and reduced hemoglobin yield, at various wave-lengths, extinction coefficients which, corrected for concentration and depth of solution, are almost identical with those obtained with 1:100 and 1:1000 dilutions of blood in 1 cm. and 10 cm. cells, respectively. This extends the range of validity of Beer's law for these pigments. Owing to turbidity, non-hemolyzed blood yields very different constants. An obvious application of the new technique is to the measurement of oxygen saturation of blood as drawn.

COMPARATIVE STUDIES IN THE SYNTHESIS OF L-ROBOSE

BY W. C. AUSTIN AND FRED L. HUMOLLER

(From the Department of Physiological Chemistry, Loyola University School of Medicine, Chicago)

A new study of the reactions l-arabinose → l-arabonolactone → l-ribonolactone → l-ribose, originally investigated by Fischer and Piloty, has been made. From 900 gm. of l-arabinose there were prepared, in improved conditions of oxidation and isolation, 660 gm. of l-arabonolactone. From the treatment of this amount of l-arabono-

lactone with aqueous pyridine at 126° there were obtained, by the method of van Ekenstein and Blanksma, \(^4\) 220 gm. of the phenylhydrazide of \(\text{D-ribonic acid, m.p. 158°}.\) This substance was converted to 104 gm. of sirupy \(\text{L-ribonolactone, all of which was subjected to reduction with the formation of 65 gm. (estimated) of \(\text{L-ribose.}\) The separation of the \(\text{L-ribose and unchanged \(\text{L-ribonolactone has been only partially successful, and has given 3 gm. of crystalline \(\text{L-ribose.}\)}}\)

These studies indicate a maximal yield of 0.5 per cent in the above method of conversion of \(\text{L-arabinose to \(\text{L-ribose, while the method of transforming \(\text{L-arabinose by way of \(\text{L-arabinal to \(\text{L-ribose, recently announced by Austin and Humoller,}\)}}\) gave a yield of 10 per cent.\)}\)

THE OXIDATION-REDUCTION POTENTIALS OF LACTATE-PYRUVATE IN THE PRESENCE OF THE ACTIVATING COENZYME OF \(\alpha\)-HYDROXYOXIDASE

BY E. S. GUZMAN BARRON AND A. BAIRD HASTINGS

(From the Lasker Foundation for Medical Research and the Department of Medicine of the University of Chicago, Chicago)

The potential of the system lactate-pyruvate has been studied in the presence of the activating coenzyme of \(\alpha\)-hydroxyoxidase derived from gonococci and a suitable reversible dye. All three components are necessary to establish a stable, reversible potential. The dye, which is reduced by the system lactate-enzyme-pyruvate,


**TABLE I**

<table>
<thead>
<tr>
<th>pH</th>
<th>(E'_1)</th>
<th>(E_0)</th>
<th>pH</th>
<th>(E'_1)</th>
<th>(E_0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.53</td>
<td>-0.092</td>
<td>+0.2453</td>
<td>7.012</td>
<td>-0.1880</td>
<td>+0.2469</td>
</tr>
<tr>
<td>5.92</td>
<td>-0.113</td>
<td>+0.246</td>
<td>7.106</td>
<td>-0.1854</td>
<td>+0.248</td>
</tr>
<tr>
<td>6.24</td>
<td>-0.1294</td>
<td>+0.2352</td>
<td>7.392</td>
<td>-0.2021</td>
<td>+0.2488</td>
</tr>
<tr>
<td>6.40</td>
<td>-0.1461</td>
<td>+0.2443</td>
<td>7.797</td>
<td>-0.229</td>
<td>+0.2466</td>
</tr>
<tr>
<td>6.699</td>
<td>-0.1655</td>
<td>+0.2431</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Average ................. +0.246
acts as a mediator in effecting the electron transfer to the electrode. With cresyl violet plus pyocyanine as mediator, ratios of lactate to pyruvate from 1:9 to 5:5 gave satisfactory potentials, indicating reversibility of the system. At these ratios the mediator was not completely reduced. When the ratio of lactate to pyruvate was 9:1, the dye was completely reduced and the potential did not reach an equilibrium value. The potential is independent of the concentration of the lactate within the limits studied. The variation of the potential with pH from 5.53 to 7.79 has been studied, as indicated in Table I. \( \frac{\Delta E}{\Delta \text{pH}} \) was found to equal 0.061. The normal potential of the system, \( E_0 \), was calculated to be +0.246 ± 0.002 and the free energy, \( \Delta F \), to be +11,630 calories.

THE AVAILABILITY OF INDOLE DERIVATIVES FOR SUPPLEMENTING DIETS DEFICIENT IN TRYP TOPHANE

BY LYLE C. BAUGUESS AND CLARENCE P. BERG

(From the Laboratory of Biochemistry, State University of Iowa, Iowa City)

Interest in the possibility of replacing essential amino acids in the diet with synthetic products more or less closely related in chemical structure has led us to synthesize \( \beta \)-3-indoleacrylic acid and \( \alpha \)-oximino-\( \beta \)-3-indolepropionic acid and feed them to rats in conjunction with a diet deficient in tryptophane. Reports in the literature on the availability of \( \beta \)-4-imidazoleacrylic acid in supplementing diets deficient in histidine are conflicting. No \( \alpha \)-oximino acid has been studied in this connection. The oxime of pyruvic acid, however, has been shown to undergo reduction, in the presence of vigorously fermenting yeast, to alanine.

The \( \beta \)-3-indoleacrylic acid was prepared by condensing \( \beta \)-3-indolealdehyde with malonic acid in the presence of pyridine and piperidine; the \( \alpha \)-oximino-\( \beta \)-3-indolepropionic acid by condensing \( \beta \)-3-indolepyruvic acid with hydroxylamine. Neither product showed any capacity to replace tryptophane for purposes of growth under the experimental conditions employed.
BACTERIA AND THE SYNTHESIS OF CAROTENE AND VITAMIN A

BY CARL A. BAUMANN, H. STEENBOCK, AND MARY A. INGRAHAM

(From the Laboratories of Agricultural Chemistry and Agricultural Bacteriology, University of Wisconsin, Madison)

By use of spectrophotometric, colorimetric, and biological methods of assay, carotenoids were found to be synthesized by many different species of bacteria. Certain bacteria produced carotene itself. The synthesis of vitamin A as such could not be demonstrated. Cultures which, on the basis of experiments with rats, were suspected of having produced vitamin A were found to have synthesized enough carotene to account for all of the vitamin A activity.

THE THYROID AND CYANIDE METABOLISM

BY EMIL J. BAUMANN, DAVID SPRINSON, AND NANNETTE METZGER

(From the Laboratory Division, Montefiore Hospital, New York)

A study of the fate of potassium cyanide, methyl cyanide, and benzyl cyanide and the relation of thyroid to their metabolism is reported. Lang has shown that about 15 per cent of acetonitrile fed to dogs could be recovered in the urine as thiocyanate. From the urine of normal rabbits we have been able to recover as thiocyanate 25 per cent or more of injected acetonitrile but only 3 to 5 per cent from thyroidectomized rabbits.

With potassium cyanide or benzyl cyanide, on the other hand, 75 per cent or more of injected cyanide can be recovered as thiocyanate from the urine of normal animals. After thyroidectomy the amount of thiocyanate excreted decreases temporarily, returning later to the preoperative level. When inorganic thiocyanates were injected into rabbits, 80 to 90 per cent could be found in the urine. The recoveries in the case of benzyl cyanide and of potassium cyanide are therefore nearly complete and are only temporarily influenced by a lack of thyroid, whereas, with methyl cyanide, a relatively small part is excreted in the urine.

This is another instance of the difficulty with which demethylation takes place in the animal body and even this limited capacity of the body to deal with demethylation depends largely on the presence of thyroid.
THE EFFECT OF FEEDING AMINO ACIDS IN CASES OF MUSCULAR DYSTROPHY

BY HOWARD H. BEARD AND CARLO J. TRIPOLI

(From the Departments of Biochemistry and Medicine, Louisiana State University Medical Center and the State Charity Hospital, New Orleans)

10 gm. of glycine were fed daily to a patient with neuromuscular atrophy of 9 months duration. 10 gm. of glutamic acid were fed daily to a patient with progressive muscular atrophy of 21 years duration and to a case of "psychopathic inferiority complex" with myatrophy from disuse. The effect of this treatment upon the creatine-creatinine metabolism is shown in Table I.

The marked initial creatinuria after the amino acid therapy indicated increased creatine formation in the body. A disappearance of this creatinuria in a few weeks indicated a retention of daily to a patient with progressive muscular atrophy of 21 years duration and to a case of "psychopathic inferiority complex" with myatrophy from disuse. The effect of this treatment upon the creatine-creatinine metabolism is shown in Table I.

The marked initial creatinuria after the amino acid therapy indicated increased creatine formation in the body. A disappearance of this creatinuria in a few weeks indicated a retention of

### Table I

**Influence of Feeding Glycine and Glutamic Acid on Creatine-Creatinine Formation in Cases of Muscular Dystrophy**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Amino acid, 10 gm. daily</th>
<th>Average 24 hr. output</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total N</td>
</tr>
<tr>
<td></td>
<td></td>
<td>g.m.</td>
</tr>
<tr>
<td>B.S., neuromuscular dystrophy</td>
<td>Control</td>
<td>11.02</td>
</tr>
<tr>
<td></td>
<td>Glycine</td>
<td>15.00</td>
</tr>
<tr>
<td></td>
<td>Increase</td>
<td>36.1%</td>
</tr>
<tr>
<td></td>
<td>Glycine</td>
<td>14.95</td>
</tr>
<tr>
<td></td>
<td>Increase</td>
<td>35.6%</td>
</tr>
<tr>
<td></td>
<td>Glycine</td>
<td>11.20</td>
</tr>
<tr>
<td></td>
<td>Increase</td>
<td>None</td>
</tr>
<tr>
<td>W.B.S., progressive muscular dystrophy</td>
<td>Control</td>
<td>13.04</td>
</tr>
<tr>
<td></td>
<td>Glycine</td>
<td>14.01</td>
</tr>
<tr>
<td></td>
<td>Increase</td>
<td>7.4%</td>
</tr>
<tr>
<td></td>
<td>Glutamic acid</td>
<td>15.95</td>
</tr>
<tr>
<td></td>
<td>Increase</td>
<td>22.3%</td>
</tr>
<tr>
<td>M.R., psychopathic inferiority complex</td>
<td>Control</td>
<td>7.24</td>
</tr>
<tr>
<td></td>
<td>Glutamic acid</td>
<td>6.90</td>
</tr>
<tr>
<td></td>
<td>Increase</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Glutamic acid</td>
<td>5.58</td>
</tr>
<tr>
<td></td>
<td>Increase</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Glutamic acid</td>
<td>6.38</td>
</tr>
<tr>
<td></td>
<td>Increase</td>
<td>None</td>
</tr>
</tbody>
</table>
creatine in the muscles. All patients are slowly recovering their strength and muscle tissue and are gaining in body weight. These findings are in agreement with results from about twenty similar cases being treated with glycine at the present time.

**A SPECTROGRAPH FOR THE RAPID QUANTITATIVE ESTIMATION OF VITAMIN A, AND DETERMINATIONS OF THE VITAMIN A CONTENT OF CERTAIN FISH OILS**

*By Charles E. Bills*

*(From the Research Laboratory, Mead Johnson and Company, Evansville, Indiana)*

A spectrograph was constructed with quartz optical parts and a replica transmission grating, so as to give two adjacent spectrograms, each 40 mm. wide by 80 mm. long. An optical wedge in front of the collimator provides means of revealing directly the intensity of the banded absorption due to vitamin A, and the unusual width of the spectra permits accurate evaluation of the bands of the standard solution and of the unknown.

The spectrograms are read by a comparison scale based on precision assays with 1000 rats on four fish oils—salmon oil (a weak source of vitamin A), cod liver oil (the standard source), halibut liver oil (a very potent source), and the liver oil of *Ophiodon elongatus*. The latter was found to be the most potent known source of vitamin A. The liver oil of *Anoplopoma fimbria* is also extraordinarily potent in vitamin A, being somewhat superior to halibut liver oil. These two fishes, under the misleading popular names of ling cod and black cod respectively, are caught in quantity off the Pacific coast.

**FURTHER STUDIES ON THE UTILIZATION OF PARENTERALLY ADMINISTERED IRON**

*By Franklin C. Bing, Esther M. Saurwein, and Victor C. Myers*

*(From the Department of Biochemistry, School of Medicine, Western Reserve University, Cleveland)*

Recently Eveleth, Bing, and Myers* reported that intraperitoneal injections of ferric chloride markedly stimulate growth, red

blood cell production, and hemoglobin formation in milk-fed anemic rats. With the parenteral method of administration the iron is effective in very small doses and, contrary to what has been found when the iron is given by mouth, copper has no effect on the rate or extent of recovery. These results were obtained with rats that never had access to solid foods in the preexperimental period and that were restricted after weaning to whole Guernsey milk containing 0.34 mg. of copper per liter. The Biazzo method, as described by Ansbacher, Remington, and Culp, was used to determine the copper.

These experiments have now been repeated with milk obtained with special precautions to avoid any metallic contamination. The copper content, as estimated by the sodium diethyldithiocarbamate method, was only 0.14 mg. per liter. With this milk it was found that severely anemic rats recovered when 0.5 mg. of iron as ferric chloride was injected into the peritoneal cavity every other day. The daily administration by mouth of 0.5 mg. of iron plus 0.025 mg. of copper was also curative.

Most of the rats also recovered when pure solutions of ferric chloride were added to the diet, but the rate of hemoglobin regeneration was slow. The cure was accelerated by increasing the dosage of iron. Several animals, however, died within a few days after treatment with 0.5 mg. of iron by mouth was begun. None of these animals, which weighed about 50 gm., drank as much as 15 cc. of milk per day during the period of oral iron administration. Apparently, anemic animals receiving 0.5 mg. of iron by mouth will recover only when they are able to drink 20 cc. or more of milk each day.

The absorption of iron apparently plays a principal part in the production of hemoglobin by anemic rats. Those procedures which aim to increase the absorption of iron, such as increasing the dosage of pure ferric chloride administered by mouth, or injecting small amounts of iron intraperitoneally, accelerate the rate of hemoglobin production. No evidence has been obtained that would lead one to conclude that copper, in addition to the small amounts present in the milk as secreted by the cow, is needed when iron is administered intraperitoneally.

SOME CHEMICAL AND PHYSIOLOGICAL PROPERTIES OF CALICREIN*

BY FRITZ BISCHOFF AND A. H. ELLIOTT

(From the Chemical Laboratory of the Potter Metabolic Clinic, Santa Barbara Cottage Hospital, Santa Barbara)

Preparations of callicrein assaying 10 K.E. units per mg. were tested for stability to acidity, to heat, and to oxidizing and reducing agents. The substance is stable at the normal acidity range of urine (pH 5.0 to 8.0). At pH 1.3, 80 per cent, at pH 4.0, 80 per cent, at pH 4.2, 70 per cent, and at pH 12.0, 80 per cent inactivation occurred in 1 hour at 20°. 5 minutes at 100° resulted in 80 per cent loss of potency; at 55° the loss was only 20 per cent. The substance was stable in aqueous H₂S, HCHO, and Na₂SO₃ solutions (0.01 M). It was inactivated by I and H₂O₂. By confining the adsorption and precipitation procedures to the conditions of stability established in the above experiments, a degree of purification to 400 K.E. units per mg. of nitrogen was attained.

Certain physiologic properties of callicrein were compared with those of various organ extracts, notably an insulin-free extract of pancreas. Callicrein dilates the coronary arteries of the perfused rabbit heart to a greater extent than the purine derivatives, but extracts of skeletal muscle, of heart muscle, of liver, and of pancreas are more vigorous coronary dilators. These organ extracts abolish ventricular fibrillation produced artificially in the perfused rabbit heart, whereas callicrein has no such effect. Neither callicrein nor pancreatic extract produces heart block in the guinea pig, a supposedly specific test for adenosine and adenylic acid.

Callicrein entirely inhibits the pressor effect of adrenalin (intravenous injection) in the rabbit and protects this animal from lethal doses. The organ extracts tested do not have this property, with the exception of pancreatic extract which does so inconstantly.

Adrenalin hyperglycemia is not influenced by callicrein.

* Presented before the American Society for Pharmacology and Experimental Therapeutics.
FURTHER OBSERVATIONS ON THE DIETARY PRODUCTION OF FATTY LIVERS IN RATS*

BY N. R. BLATHERWICK, E. M. MEDLAR, PHOEBE J. BRADSHAW, ANNA L. POST, AND SUSAN D. SAWYER

(From the Biochemical Laboratory and the Hegeman Memorial Laboratory of the Metropolitan Life Insurance Company, New York)

We reported previously the occurrence of fatty livers in rats fed diets containing whole liver. Experiments showed that a liberal allowance of carbohydrate in the diet does not prevent the deposition of fat in the liver. It was also reported that feeding a dried 70 per cent alcoholic precipitate of an aqueous extract of liver does not cause the livers to become fatty. It was also found that the feeding of residues from aqueous and from alcoholic extraction of liver does not produce fatty livers.

This investigation has been continued with the following results. The addition of 0.1 per cent of desiccated thyroid to a diet containing 75 per cent of dried, whole liver has no effect on the deposition of fat in the liver. When 1.7 per cent of egg lecithin is added to diets containing dried, whole liver, there is no marked effect in reducing the fat content of the liver. Such diets naturally contain large amounts of cholesterol and lecithin. Fatty livers may be easily produced by adding 1.0 per cent of cholesterol to diets containing liver fractions which are ineffective without the cholesterol. The addition of 1.7 per cent of egg lecithin to such diets is sometimes without effect and at other times it appears to reduce this fatty condition. It has been found that fatty livers occur when rats are fed diets consisting entirely of cooked egg yolks or of cooked whole eggs. The greater deposition of fat is found when the whole eggs are eaten. These two diets are somewhat comparable to the liver diets with respect to their content of cholesterol and lecithin. The blood of rats having fatty livers usually has a normal lipid content. The fatty acids of the abnormal livers appear to be those normally present.

* Presented before the American Society for Experimental Pathology.

A FORMALDEHYDE-STABLE, PROTEOLYTIC BACTERIAL ENZYME

By Alden Kinney Boor and C. Phillip Miller
(From the Department of Medicine of the University of Chicago, Chicago)

When to a suspension (equivalent to 0.25 per cent dried organisms) of certain bacteria formaldehyde was added in appropriate concentration, the rate of acid production was found to be greatly accelerated. The phenomenon was observed in the case of two strains each of Micrococcus catarrhalis and meningococcus and one of four strains of gonococcus studied. It was lacking in the case of a single strain each of pneumococcus, staphylococcus, and hemolytic streptococcus.

The optimum concentration of formaldehyde was 27 mm per liter. At higher concentrations the rate of acid production was not so great, and at 135 mm no acid was formed.

When the reaction of the suspension was maintained at pH 8.2, acid formation was inhibited. The maximum acidity developed by the suspension without adjustment with NaOH was pH 3.2, which was reached at the end of 4 days. The acidity developed most rapidly at temperatures between 37-50°. Complete inhibition of the reaction was accomplished by treating the bacterial suspension (before the addition of formaldehyde) with 4 mols of ethyl alcohol for 2 hours, or by heating at 60° for 15 minutes, 75° for 5 minutes, or boiling for 1 minute. The salts of mercury, copper, and silver, in small concentrations, were inhibitory. Glycerol extracts were inactive.

The reaction was increased by the addition of egg white, casein, or peptone to the bacterial suspensions.

HEMATOPORPHYRIN, AN ARTIFICIAL PROTEOLYTIC ENZYME

By M. J. Boyd
(From the Laboratory of Biochemistry, University of Cincinnati, Cincinnati)

Hematoporphyrin in the presence of visible or ultra-violet light and oxygen digests fibrinogen quickly; serum albumin, slowly; edestin, very slowly or not at all. The striking fact is that oxygen is necessary for the proteolytic action of this artificial enzyme, for if oxygen be replaced by hydrogen, very slight or no digestion takes place. The fibrinogen is converted into hydrolytic products.
which are similar to those produced by the action of thrombin and rattlesnake venom on fibrinogen. This artificial system, which resembles digestive enzymes in action, supports the theory of Dr. A. P. Mathews that enzymes are conductors of energy.

INHIBITION OF PEPSIN BY MUCIN

By H. C. BRADLEY

(From the Laboratory of Physiological Chemistry, University of Wisconsin, Madison)

The effective clinical use of mucin in cases of gastric ulcer has raised the question of its behavior in gastric digests. Commercial mucin, prepared from hog stomachs, is a crude mixture from which considerable amounts of amino acids and peptides may be dialyzed. The highly viscous protein material remaining is almost completely resistant to peptic hydrolysis. When added to peptic digests of finely divided fibrin, it exerts a marked inhibiting effect on the digestion of fibrin. This inhibitory effect is most pronounced during the early period of digestion, and in optimal pH range. Thus at pH 1.3 fibrin alone digests completely in 5 hours. With mucin present it is 50 per cent digested in 5 hours; completely, in 24 hours. At pH 2.0 fibrin alone digests in 6 hours. With mucin present it is 50 per cent digested in 6 hours, and only 75 per cent digested in 3 days. At this level there is considerable permanent inhibition. At pH 3 fibrin alone is digested very slowly, and the addition of mucin does not materially alter this speed. The results seem to indicate adsorption of the pepsin, thus reducing its effective concentration in the digestion mixture.

FURTHER STUDIES ON THE ADMINISTRATION OF GLYCINE IN MUSCULAR AND NEUROMUSCULAR DISEASES*

By ERWIN BRAND and MEYER M. HARRIS

(From the Departments of Chemistry and of Internal Medicine, New York State Psychiatric Institute and Hospital, New York)

Metabolic studies have been carried out on a group of thirty-six patients with various muscular and neuromuscular diseases for diagnostic and therapeutic purposes. Out of this group, nine cases

* Aided by a grant from The Chemical Foundation, Inc.
of progressive muscular dystrophy have received varying amounts of glycine (7.5 to 25 gm. daily per os) for a prolonged period of time (2 to 9 months). These patients ranged in age from 8 to 24 years and presented various grades of severity of the disease. Six of these patients have been kept on weighed, meat-free, low purine diets and their urine has been collected daily for analysis. The three other patients, treated at home, have been kept under similar dietetic conditions and their urine has been examined at regular intervals.

The metabolic effects of the prolonged administration of glycine were similar to those previously reported for shorter periods. The creatine excretion rose promptly and was maintained at this higher level throughout the glycine feeding period. The creatinine excretion, however, remained practically unchanged.

The favorable therapeutic effects of glycine feeding reported by Thomas, Milhorat, and Techner in some of their cases of muscular dystrophy have not been noted in our muscular dystrophy patients up to the present time. One patient (child, age 12 years), in spite of 2 months of glycine (7.5 gm. daily) therapy suddenly became more disabled (stopped walking). None of this group experienced the muscle sensations from glycine described by these investigators. Three of our recent cases, however, have noted such sensations.

Boothby has treated several cases of muscular dystrophy with glycine and indicates that there was only questionable if any improvement. However, in cases of myasthenia gravis, he and

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8 We are indebted to Drs. E. G. Zabriskie, A. M. Frantz, and C. C. Hare of the Neurological Institute for carefully examining these patients and confirming the diagnosis.

10 The glycine was supplied to us by the Calco Chemical Company, Inc., of Bound Brook, New Jersey, at a specially low price for these studies. They also started quantity production upon our suggestion.


other investigators\textsuperscript{16} report marked improvement with glycine therapy. At our suggestion a case, diagnosed as myasthenia gravis, was treated with 15 gm. of glycine daily for a period of 3 months (July to October, 1932) without any apparent improvement.

The variable results thus far obtained with glycine therapy by different investigators are probably due to difficulties in differentiating the various types of muscular diseases. It is possible that even in cases which appear quite similar clinically, the pathogenesis may be different.

In this connection, we should like to call attention to the fact that only two (Cases 1 and 3) out of the eleven cases of muscular dystrophy reported by Milhorat\textsuperscript{13} with case histories, showed definite improvement upon glycine administration. It is worthy of note that in these two cases, before the experiment was started, the average daily excretion of creatine was only 0.02 and 0.03 gm. respectively on a creatine-free diet. Considering the fact that in these cases the disease had been present for 14 and 15 years and that it was associated with appreciable physical disabilities, the almost negligible degree of creatinuria would seem most unusual for progressive muscular dystrophy. This would lead one to suspect that the cases perhaps belong to a special clinical group which responds to glycine treatment.

For this reason, one should, perhaps, venture the suggestion that these two cases and the few cases of muscular dystrophy with little or no creatinuria reported in the literature\textsuperscript{16} may belong in one group together with the cases of myasthenia gravis which have responded to glycine therapy.

In previous communications,\textsuperscript{11} we had indicated that glycine had a sparing effect upon nitrogen and sulfur metabolism. It was also pointed out that the tissues of the body, rich in glycine (e.g. connective tissue and bone), might therefore be important sources of reserve protein when increased demands are made upon the body. Upon this basis, for example, we had proposed, several years ago (1928), to treat cases of Graves' disease. We hope to be able to do so soon through the courtesy of Professor W. W. Palmer of the College of Physicians and Surgeons, Columbia University.


\textsuperscript{13} Nedelmann, E., \textit{Münch. med. Woch.}, 70, 800 (1923).
We are indebted to the Research Committee of the Neurological Institute for their cooperation.

APPLICATION OF THE LORENZ-PREGL TECHNIQUE FOR THE DETERMINATION OF SMALL AMOUNTS OF PHOSPHORUS IN BIOLOGICAL MATERIAL

BY RICHARD O. BROOKE AND ARTHUR H. SMITH
(From the Department of Physiological Chemistry, Yale University, New Haven)

The available methods for the determinations of phosphorus fall roughly into three classes: the macrogravimetric, requiring three separate precipitations and occupying a period of 3 days; the volumetric, a speedier but far less accurate method; and the colorimetric procedures that are used only when it is impossible to avoid them. It is with difficulty that the accuracy of colorimetric technique is kept within ±5 per cent.

The present report deals with an adaptation of the Lorenz-Pregl procedure whereby, in approximately 7 hours, as many as twelve gravimetric determinations can be made simultaneously on samples containing less than 0.5 mg. of phosphorus, with an accuracy of ±1 per cent.

The material is digested with sulfuric acid and nitric acid and the phosphorus in the entire sample or in an aliquot of the digest weighed as ammonium phosphomolybdate. For the precipitation, drying, and weighing of the yellow precipitate, we have used the apparatus and have followed closely the technique described by Pregl. However, in order to carry on a number of determinations simultaneously, individual air-tight containers are employed, in which the filter funnels are placed as each one is ready for dehydration. In this way, six or more are placed in a vacuum desiccator and subjected to suction at the same time. Each precipitate has therefore been exposed under precisely the same conditions.

The funnels are readily prepared for future use by dissolving the ammonium phosphomolybdate with dilute ammonia water. This is followed by a few cc. of sulfuric-chromic acid mixture and about a liter of distilled water. Each funnel is fitted to a large suction flask and connected to a reservoir in such a way that a constant stream of water is drawn slowly through. The prepara-
tion of a dozen or so filter funnels becomes automatic and requires little attention.

Tables I and II indicate the accuracy and applicability of the method.

**TABLE I**
Estimation of Phosphorus in Known Solution of Monopotassium Phosphate

<table>
<thead>
<tr>
<th>Weight of precipitate (gm.)</th>
<th>Phosphorus mg.</th>
<th>Theoretical</th>
<th>Found mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0565</td>
<td>0.818</td>
<td></td>
<td>0.821</td>
</tr>
<tr>
<td>0.0564</td>
<td>0.819</td>
<td></td>
<td>0.819</td>
</tr>
<tr>
<td>0.0566</td>
<td>0.818</td>
<td></td>
<td>0.822</td>
</tr>
<tr>
<td>0.0564</td>
<td>0.818</td>
<td></td>
<td>0.819</td>
</tr>
<tr>
<td>0.0564</td>
<td>0.818</td>
<td></td>
<td>0.821</td>
</tr>
</tbody>
</table>

**TABLE II**
Satisfactory Recovery of Phosphorus (0.409 Mg.) Added to Bone and Casein

<table>
<thead>
<tr>
<th>Material</th>
<th>P in sample mg.</th>
<th>P recovered mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone</td>
<td>0.138</td>
<td>0.412</td>
</tr>
<tr>
<td></td>
<td>0.286</td>
<td>0.411</td>
</tr>
<tr>
<td></td>
<td>0.449</td>
<td>0.411</td>
</tr>
<tr>
<td></td>
<td>0.318</td>
<td>0.411</td>
</tr>
<tr>
<td></td>
<td>0.245</td>
<td>0.410</td>
</tr>
<tr>
<td></td>
<td>0.123</td>
<td>0.406</td>
</tr>
<tr>
<td></td>
<td>0.514</td>
<td>0.408</td>
</tr>
<tr>
<td>Casein (crude)</td>
<td>0.392</td>
<td>0.410</td>
</tr>
<tr>
<td>&quot; (low ash)</td>
<td>0.397</td>
<td>0.412</td>
</tr>
</tbody>
</table>

**RELATION OF IODINE LEVEL TO FAT DEFICIENCY AND METABOLISM**

By GEORGE O. BURR AND J. H. BEBER

(From the Department of Botany, University of Minnesota, Minneapolis)

A study was made of the effect of levels of intake of potassium iodide by rats suffering from fat deficiency. It is shown that the iodine level probably plays no part in the production of fat deficiency symptoms.
THE RATE OF CHANGE OF ALKALI RESERVE AFTER INGESTION OF SALTS OF ORGANIC COMPOUNDS

By JANE CAPE

(From the Department of Physiological Chemistry, Medical School, University of Wisconsin, Madison)

In a study made to ascertain the rate of change of alkali reserve after ingestion of salts of organic acids it was first necessary to determine normal variations of the acid-base equilibrium under basal conditions, as a control measure. Observations were made on seven normal individuals during the period from 7 a.m. to 12 noon. Blood samples were taken at hourly intervals for determination of the serum pH, CO₂ content, and total base.

The CO₂ content and total base tended to run higher in all seven normals than has been reported by other investigators excepting Earle and Cullen,¹⁷ whose data for CO₂ values were confirmed. In no case did the CO₂ of the serum rise more than 4 volumes per cent during the entire observation for any one individual. The levels for all three values, pH, CO₂, and total base, in these normal individuals studied under basal conditions showed no significant variations over a morning period of 5 hours.

A further series of experiments was made on twelve normal subjects under basal conditions to determine the effect of ingesting 6 gm. of sodium citrate. The blood was collected at half hour intervals up to 90 minutes, at which time the observations were discontinued. In another series of eight subjects observations were made on the effects of the equivalent amount (5 gm.) of sodium bicarbonate. The amounts of salts given were based on the common therapeutic doses.

The maximum response occurred at times varying from 30 to 90 minutes with the different individuals. The rise in alkali reserve after the ingestion of sodium citrate was slight, the action brief, and the values tended to approach the initial preingestion level at the end of the 90 minute period. Similar effects on the alkali reserve were observed from ingestion of sodium bicarbonate.

It appears that the influence of a 6 gm. dose of sodium citrate or of a 5 gm. dose of sodium bicarbonate upon the acid-base condition of the blood is neither prolonged nor marked.

THE PREPARATION OF THYROGLOBULIN

By J. W. CAVETT and S. R. SELJESKOG
(From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis)

The thyroid glands are frozen and sliced three times. 100 gm. of gland are extracted overnight with 300 cc. of isotonic saline solution.

The filtrate from the above is adjusted to pH 7.4 and the protein fractionated with ammonium sulfate. The fraction obtained between 35 and 45 per cent saturation is retained. This precipitate of thyroglobulin is dissolved in water and precipitated at -5° with 4 volumes of acetone. The precipitate is collected on a filter, in the refrigerator, and washed five times with acetone to remove water. It is then transferred to a large centrifuge tube and washed five times by centrifugation with cold ethyl ether which is freshly distilled from sodium bisulfite. This removes most of the acetone from the protein.

The precipitate is placed in a Soxhlet extractor and extracted for 40 hours with ethyl ether. Metallic sodium placed in the flask containing the ether removes traces of acetone or water which are extracted and prevents denaturation of the thyroglobulin. The protein is dried in a vacuum desiccator. It is dissolved in water by adjusting the pH to 7 with sodium hydroxide. The acetone precipitation and ether extraction are repeated as above. Attempts at crystallization of the purified thyroglobulin have been unsuccessful. For analysis the lipid-free thyroglobulin thus obtained is placed in water, heat-coagulated, and the water changed until it becomes ammonia-free. The sample is dried on a steam bath.

A STUDY OF PROGRESSIVE PSEUDOHYPERTROPHIC MUSCULAR DYSTROPHY IN CHILDREN AFTER THE ADMINISTRATION OF GLYCYNE AND CREATINE

By ALFRED CHANUTIN, H. R. BUTT, and L. T. ROYSTER
(From the Laboratory of Physiological Chemistry and the Department of Pediatrics, University of Virginia, University)

Five cases in a family giving a history of progressive muscular dystrophy for five generations are being studied. The earliest symptoms have been detected in a child of 2 years and the severest form of the disease was seen in two cousins, aged 12 years.
One boy (B. S.), aged 6 years, was chosen for a study of his metabolism during glycine (Calco) administration. This child walked on his toes and was unable to rise from a bending position without "crawling up" his legs. After 2 weeks of glycine administration this boy was able to walk normally and rise from a bending position without assistance. The typical complaints of pain and formication as first described by Thomas and coworkers were noted. The creatine and creatinine output were only slightly affected.

Two brothers, aged 9 and 12, were given creatine at home. Their metabolism was not studied. The complaint of pain and formication was made after the 1st week's administration. An improvement in appearance of muscular ability was definitely noted after the 1st month's administration. After 4 months of creatine ingestion, however, these subjects are becoming progressively worse in appearance and in their ability to perform work.

A cousin (C. B.), aged 12, was admitted for a metabolic study during creatine administration. A decided improvement was noted in a relatively short time. The excretion of creatine and creatinine has been studied during a 3 month period. Changes in the creatine metabolism have been noted after administration of glycine to this creatine-fed subject. (The experimental data cannot be fully interpreted at this time.)

THE ABSORPTION OF LEUCINE, VALINE, AND THEIR ISOMERS FROM THE GASTROINTESTINAL TRACT OF THE WHITE RAT

By BARBARA W. CHASE

(From the Department of Physiological Chemistry, Medical School, University of Michigan, Ann Arbor)

The rates of absorption of leucine, valine, and their isomers from the gastrointestinal tract of the young white rat have been studied by the method of Cori as previously used in this laboratory.\(^{18}\) The rate of absorption of the sodium salt of \(l\)-leucine over a 3 hour period was similar to that previously obtained (approximately 43 mg. or 0.328 milli-equivalents per hour per 100 gm. of rat). The rate of absorption of the sodium salt of \(d\)-isoleucine (the naturally occurring form) was lower than that of the salt of \(l\)-leucine when fed in approximately the same concentration.

The absorption of the sodium salt of the \( dl- \) or \( d- \) valine was more rapid than that of the sodium salt of \( l- \) leucine and little difference was observed in the rates of absorption of the sodium salts of the naturally occurring \( (d-) \) valine and the \( dl \) form. The sodium salt of \( dl-\alpha\)-amino-\( \alpha \)-methylbutyric acid (isovaline) was absorbed much less readily than was the corresponding salt of \( dl \)-valine. The rate of absorption of the sodium salt of isovaline was much less than that of the free amino acid.

**THE ELECTRICAL FORCES IN SYSTEMS CONTAINING BIOLOGICAL COMPONENTS**

**II. MOLAL VOLUMES OF AMINO ACIDS, PROTEINS, AND CERTAIN RELATED SUBSTANCES**

By Edwin J. Cohn, Thomas L. McMeekin, John T. Edsall, and M. H. Blanchard

(From the Department of Physical Chemistry in the Laboratories of Physiology, Harvard Medical School, Boston)

Traube (1899) attempted to devise a system of atomic volumes in terms of which molecular volumes of organic compounds could be calculated in the polar solvent, water. The increment in molal volumes of alcohols and aliphatic acids yielded 16.1 for the CH\(_2\) group. Comparable studies yielded 18.9 for the carboxyl, 5.4 for the hydroxyl, and 7.7 for the amino group. From this the atomic volume of carbon was deduced to be 9.9, of hydrogen 3.1, and of nitrogen 1.5. Calculations made with this series of atomic volumes always resulted, however, in estimated molecular volumes too small by approximately 12 cc. per mol.

**Amino Acids**—The apparent molal volumes of the amino acids may, however, be calculated with considerable accuracy from the atomic volumes of Traube. In this respect amino acids may be contrasted with other known organic compounds. Thus the molal volumes of aliphatic \( \alpha \)-amino acids are:

<table>
<thead>
<tr>
<th></th>
<th>Glycine</th>
<th>Alanine</th>
<th>Valine</th>
<th>Leucine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calculated</td>
<td>42.7</td>
<td>58.8</td>
<td>91.0</td>
<td>107.1</td>
</tr>
<tr>
<td>Observed</td>
<td>43.5</td>
<td>60.6</td>
<td>91.3</td>
<td>107.5</td>
</tr>
</tbody>
</table>

\(^{19}\) Nitrogen was assumed to vary somewhat, and oxygen even more, depending upon its valence and its position in the molecule (Traube, J., *Samml. chem. u. chem.-techn. Vortr.*, 4, 255 (1899)).
The tighter packing of amino acids is believed to depend upon electrostriction of solvent molecules due to the Zwitter Ions, and to have profound physiological significance. As evidence for this view, and for the fortuitous nature of the agreement between these calculations and observations, may be cited the still smaller apparent molal volumes of amino acids of greater dipole moments, isomeric with alanine, valine, and leucine: β-alanine, 58.8, γ-aminovarlic acid, 90.2, ε-aminocaproic acid, 104.9. The greater the electrostatic forces surrounding the molecule, the greater the electrostriction and the smaller the apparent molecular solution volume.

Amino Acid Compounds—Destruction of the Zwitter Ions nature of amino acids increases their molecular volumes. The formyl derivatives of glycine and leucine have been studied as well as hydantoin and leucine hydantoin. These compounds of amino acids are not Zwitter Ions, but weak acids. As such they are far more soluble in alcohol than amino acids, and like other organic compounds, their molecular volumes are greater than those calculated from Traube's atomic volumes.

<table>
<thead>
<tr>
<th></th>
<th>Molecular solution volumes</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calculated</td>
<td>Observed</td>
</tr>
<tr>
<td>Ethyl alcohol</td>
<td>40.7</td>
<td>52.8</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>38.1</td>
<td>50.7</td>
</tr>
<tr>
<td>Hydantoin</td>
<td>56.1</td>
<td>64.7</td>
</tr>
<tr>
<td>Formylglycine</td>
<td>58.1</td>
<td>70.6</td>
</tr>
<tr>
<td>Hydantoin of leucine</td>
<td>120.5</td>
<td>131.5*</td>
</tr>
<tr>
<td>Formylleucine</td>
<td>122.5</td>
<td>136.8</td>
</tr>
<tr>
<td>Urea</td>
<td>30.8</td>
<td>44.3</td>
</tr>
</tbody>
</table>

* Because of the insolubility of the hydantoin of leucine in water, these measurements were made in 80 per cent alcohol. The values of uncharged molecules are generally larger in non-polar solvents; thus formylglycine is 70.6 in water and 72.4 in 80 per cent alcohol.

These results also lead us to associate the tighter packing in amino acid solutions with their charged condition, and suggest a criterion of Zwitter Ion structure.

Urea—Urea, like the amino acids, increases the dielectric constant of water. It has accordingly been suggested that it was
Zwitter Ion in nature, a view opposed by Ebert. On the basis of the above criterion the molecular solution volume of urea (see tabulation above) demonstrates that it does not exist predominantly as a Zwitter Ion. The ratio of its solubility in water to that in alcohol \( \frac{N_{\text{CH}_3\text{OH}}}{N_{\text{H}_2\text{O}}} = 0.1794 \) is comparable to that of hydantoin (0.2589) and formylglycine (0.4567) rather than to glycine, being smaller because of its smaller molecular volume. Glycine, with the same molal volume as urea, has a ratio, smaller than 0.001, comparable to electrolytes rather than uncharged molecules. The solubility ratio, previously employed as a criterion of the electrostatic forces surrounding these molecules, thus contrasts the behavior of the amino acids and urea, precisely as does the criterion of molecular solution volumes, leaving no doubt that urea is not to be regarded as a Zwitter Ion.

Proteins—Proteins are no less Zwitter Ions than the amino acids of which they are composed. Their dipole moments presumably differ widely, depending upon the number and distribution of charges upon their molecules. Solubility reflects this distribution, increasing with the dipole moment and diminishing with the molecular volume. The molecular volumes of most proteins are three-fourths of their molecular weights, since their specific volumes are roughly the same, 0.73 to 0.76. An exception is gelatin, 0.682, which contains 25 per cent glycine. Zein contains as much of the largest amino acid, leucine, as gelatin of the smallest. Its specific solution volume is 0.76 and that of edestin, with a more normal amino acid distribution, 0.744. The respective specific solution volumes calculated from those of the amino acids they contain, allowance being made for the water lost in peptide linkage, are: gelatin 0.69, edestin 0.73, zein 0.76.

Denatured Proteins—The solution volumes of uncharged organic molecules are greater in alcohol and in benzene than in water. The solution volumes of amino acids and proteins decrease in alcohol-water mixtures, due to increased electrostatic forces.

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20 Devoto, G., *Gazz. chim. ital.*, 60, 520 (1930); 61, 897 (1931).
tured proteins are less soluble than native proteins. The hypothesis is suggested that in denaturation amino and carboxyl groups are brought sufficiently close together for their positive and negative charges to come within the range of each other's attraction spheres. A smaller dipole moment would result, and a partial loss of Zwitter Ion properties. When these are regained, the attraction between the positive and negative groups is first broken by the formation of protein salts, followed by their decomposition, and regeneration of the Zwitter Ion at its isoelectric point.

**Biological Environment**—The small apparent molecular volumes and high solubility of amino acids and proteins of large dipole moments are associated with the orientation and the close packing of solvent and solute molecules. Further, the high dielectric constants of systems containing such components result in mutual solvent action and in reactions with electrolytes which conform approximately to ideal solution laws, even in the concentrated matrix, which is the biological environment.

**PHYSIOLOGICAL PROPERTIES OF THE ANTERIOR PITUITARY-LIKE HORMONE**

By J. B. Collip, D. L. Thomson, and Hans Selye
(From the Department of Biochemistry, McGill University, Montreal, Canada)

Female rats receiving large doses of the anterior pituitary-like hormone of the human placenta show enlarged ovaries with large corpora lutea and follicular cysts; the anterior pituitary is also enlarged and there is evidence of thyroid hyperactivity. These effects cannot be maintained over long periods; in males and castrates, the pituitary and thyroid are not affected. The mammary glands undergo great development in intact virgin rats so treated, and actual secretion of milk may then be induced by castration. In hypophysectomized male rats, the hormone leads to increase of interstitial tissue and enlargement of accessory organs without checking degeneration of the germinal epithelium. In prepubertal hypophysectomized females, or in intact infantile females, it leads to formation of thecal luteinization; in adult hypo-

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physectomized females, it leads to continuous estrus from the shrinking ovary.

PHYSIOLOGICAL PROPERTIES OF CERTAIN PITUITARY EXTRACTS

BY J. B. COLLIP, D. L. THOMSON, AND HANS SELYE
(From the Department of Biochemistry, McGill University, Montreal, Canada)

Methods are described for the preparation of various extracts from the anterior pituitary of beef, sheep, and pig. With suitable extracts, or combinations of extracts, it has been possible to obtain follicular development and formation of fresh corpora lutea in the ovaries of hypophysectomized rats, and rapid growth in hypophysectomized rats.

FERMENTABLE SUGAR IN HEART AND SKELETAL MUSCLE

BY GERTY T. CORI AND JOHN O. CLOSS
(From the Department of Pharmacology, Washington University School of Medicine, St. Louis)

Muscle was either frozen and cut into thin slices, or submerged directly, without freezing, in ice-cold 0.5 N H₂SO₄ and ground very thoroughly with sand. After precipitation with HgSO₄ and BaCO₃ reducing power was determined before and after yeast fermentation. The following values (in mg. per cent) were obtained. At true plasma sugar levels of 133 to 183 fermentable sugar of rabbit heart ranged from 46 to 64. This is considerably higher than fermentable muscle sugar values, which varied between 16 and 33, at comparable plasma sugar levels. At plasma sugar levels of 56 to 78 (i.e., after insulin injection) heart sugars varied between 14 and 26, while muscle sugar was reduced to vanishing proportions. During hyperglycemia, heart muscle was also found to contain more sugar than skeletal muscle. Rats fasted for 24 hours showed muscle sugars of 10 to 12 at plasma sugar levels of 80 to 120. Frog muscle contained the same amount of fermentable sugar, though the plasma sugar was only around 25. Short tetanic stimulation of rat muscle in situ or of excised frog muscle resulted in an increase in fermentable muscle sugar to twice the normal value or more. The rate of fermentation of the sugar of resting and of stimulated muscle was similar to that of glucose.
PROTEINS OF YEAST (SACCHAROMYCES CEREVISÆ)

By FRANK A. CSONKA

(From the Protein and Nutrition Division, Bureau of Chemistry and Soils, United States Department of Agriculture, Washington)

When studying the nutritive value of proteins by feeding experiments, the possible effect of the protein present in the yeast, which is added to supply certain vitamins, is generally disregarded by investigators. Information on the amino acid composition of yeast proteins is meager. Dreyer, in 1913, claimed that yeast contains two proteins, an albumin and a globulin. He used strong alkaline solvents to extract the proteins, a procedure which should be avoided, if possible, because of the danger of formation of secondary products.

Ether treatment of the yeast previous to extraction with water and 10 per cent salt solution made it possible to extract 60 per cent of the total yeast nitrogen. The yeast protein was prepared both by coagulation and by acidification methods applied to the salt extract. It is precipitated by ammonium sulfate at 70 to 80 per cent saturation. It is not precipitated by dilution nor by dialysis of the salt extracts because of the presence of proteolytic enzymes, which decompose the protein rapidly on standing. A study of the physical behavior and amino acid composition of the yeast protein is under way.

A RAPID METHOD FOR THE PREPARATION OF THEELIN

By JACK M. CURTIS

(From the Laboratory of Biological Chemistry, St. Louis University School of Medicine, St. Louis)

Zondek has called attention to the presence of large quantities of estrus-producing material in the urine of pregnant mares. He found that hydrolysis with acid was necessary to render the compound soluble in ether. Though several reports on the nature of the compounds of mare urine have appeared, no method of extraction and purification of this material has been described. The following rapid method has been used successfully.

1. Acidification and Precipitation—(a) Urine is acidified with

HCl to pH 1 (approximately) and kept at room temperature for 1 week. (b) 5 to 15 gm. of sodium benzoate per liter of urine are added. (c) The urine is filtered and the precipitate dried.

2. Ethyl Ether Extraction—(a) The precipitate is extracted with ethyl ether. (b) Wash the ether solution with strong NaOH until the pH of the aqueous phase is 8.5 to 9. (c) Extract the aqueous washings with ether. (d) The ether extracts are combined and distilled.

3. Distribution between Butyl Alcohol-Petroleum Ether and Aqueous Alkali—(a) The residue from (2-d) is taken up in equal parts of butyl alcohol and petroleum ether and this solution is washed with Na$_2$CO$_3$. (b) The estrus-producing material is extracted from organic solvents with dilute alkali. (c) The alkaline solution is acidified and extracted with ether. (d) Ether is distilled.

4. Semicarbazone Purification—(a) The residue from (3-d) is dissolved in ethyl alcohol and treated with semicarbazide. (b) Semicarbazone is recrystallized from ethyl alcohol; hydrolyzed. (c) Theelin is recrystallized from aqueous alcohol; m.p. 251–252° (uncorrected).

This method of purification is remarkably simple when compared with the methods used to purify the material from urine of pregnant human cases. There are only two distillations, both of ether extracts, in the four essential steps. The actual time for the preparation of crystalline theelin from 5 gallons of urine should not exceed over 10 hours.

The yield of theelin is large, making it practicable to work up the urine to 5 to 10 gallon batches, eliminating the necessity of handling large volumes of urine. From 10 gallons of urine it has been possible to isolate over 1 gm. of theelin having a melting point of 251–252°.

NITROGEN CONSERVATION AND HEMOGLOBIN CONSTRUCTION AS INFLUENCED BY IRON SALTS IN ANEMIC DOGS

BY FLOYD SHELTON DAFT

(From the Department of Pathology, The University of Rochester School of Medicine and Dentistry, Rochester, New York)

Whipple and Robscheit-Robbins$^{27}$ showed that anemic dogs on a diet devoid of nitrogen could be caused, by the administration

of iron salts, to regenerate considerable amounts of hemoglobin. These authors were led to believe that this indicated a conservation of nitrogenous material which otherwise would be wasted.

The results of the experiments to be reported indicate very strongly that this explanation is correct. There is very good evidence of conservation of nitrogenous material which normally would be completely catabolized. Although no definite statement can be made on this point, there is no evidence that the iron salts cause an increase in the breakdown of tissue protein. There is some evidence that the anemic dog will break down more protein than the non-anemic dog under identical conditions.

Anemic dogs were kept for 3 weeks on a diet of sugar. During the last 2 weeks of this period ferric citrate, equivalent to 400 mg. of iron, was fed daily. The total urinary output of ammonia, urea, creatinine, creatine, uric acid, and of total nitrogen was determined. Control values were secured by conducting identical experiments on the dogs before they were made anemic or after normal hemoglobin levels had been restored. Further control values were obtained by experiments, on anemic dogs, in which iron salts were not added to the diet of sugar.

During the feeding of ferric citrate to the anemic dogs there was a diminution in the output of nitrogen, principally in the urea fraction. The authors interpret this as indicating a conservation of nitrogenous material, which, were it not for the anemic condition and the administration of the iron salts, would be completely catabolized.

THE SEXUAL VARIATION IN THE GLYCOGEN AND FAT CONTENT OF THE LIVER

By HARRY J. DEUEL, Jr.

(From the Department of Biochemistry, University of Southern California School of Medicine, Los Angeles)

A difference in carbohydrate metabolism must exist between the sexes since it has been shown that women develop a much more rapid and pronounced ketonuria during fasting than do men. Moreover, a similar variation has been found in rats and guinea pigs during fasting if diacetic acid is administered. In order to

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determine whether these discrepancies were associated with a lower carbohydrate reserve in the female animals, a determination of the glycogen and fat content of the liver and of the glycogen in the muscle was made in rats and guinea pigs. So that any discrepancies which might be attributed to a variability in the amount of food in the gastrointestinal tract at the beginning of the fast might be obviated, the animals in most cases underwent a preliminary fast of 48 hours, after which glucose was administered in 50 per cent solution by stomach tube in a dose of 0.500 gm. per 100 sq. cm. of body surface. The animals were killed after a subsequent fast of 24, 36, 48, or 72 hours, amytal being used as an anesthetic, and the liver and muscle samples were removed. Normal male rats have a higher glycogen content in the liver and a lower percentage of fat than do normal female animals. No definite sexual variability in liver glycogen could be demonstrated in guinea pigs (probably due to a rapid glycogenolysis before the liver was removed, since we found high blood sugar values) but the fat content of this organ was invariably much higher than in the male guinea pigs. Ovariectomized rats gave results comparable with those of normal males, while the castrate females which received sufficient theelin to keep them in active estrus had glycogen stores comparable with normal females. No sexual differences in liver glycogen were observed in sexually immature rats. It is concluded that the difference in ketolysis which obtains between the sexes may be traced to a smaller glycogen reserve in the females, probably as a result of a lower supply of ketolytic precursors.

SPECTROPHOTOMETRIC STUDIES OF HEMOGLOBIN DERIVATIVES

BY DAVID L. DRABKIN AND J. HAROLD AUSTIN
(From the Department of Physiological Chemistry and the John Herr Musser Department of Research Medicine, School of Medicine, University of Pennsylvania, Philadelphia)

We have extended our spectrophotometric studies to certain hemoglobin derivatives which have not thus far received sufficient study by this technique.

The absorption curves of hemin crystals (dissolved in 0.01 N NaOH) either oxidized in air or reduced with Na₂S₂O₄ are characteristic though flat and ill defined. Reduced hemin in alkaline solution combines with various nitrogenous substances to form
reduced hemochromogens which have an exceedingly intense, narrow region of absorption in the green (the \( \alpha \) band) and a less intense and broader absorption towards the blue end of the spectrum (the \( \beta \) band). Reduced hemochromogens are easily oxidized in air, yielding oxidized hemochromogens which also have characteristic spectra with the bands shifted toward the red and with a reversal in the strength of the \( \alpha \) and \( \beta \) bands.

We have devoted most of our attention to the following derivatives: pyridine hemochromogen prepared by the addition of pyridine (final concentration 8 per cent) to reduced hemin in 0.01 \( \text{N} \) \( \text{NaOH} \); globin hemochromogen prepared by the addition of \( \text{NaOH} \) (final concentration 0.01 \( \text{N} \)) to reduced hemoglobin; and the substances formed by the addition of pyridine to reduced hemoglobin or to oxyhemoglobin.

The last mentioned derivatives seem of especial interest. On the addition of pyridine to reduced hemoglobin a characteristic orange-red precipitate is formed when the concentration of pyridine is 8 per cent; upon further addition of pyridine to a concentration of 40 per cent the precipitate dissolves, yielding a solution with a characteristic reduced hemochromogen-like spectrum. This solution saturated in a tonometer with CO undergoes a change in color and the spectrum developed is very similar to, though not identical with, that characteristic of CO hemoglobin. Removal of CO by evacuation restores the original reduced hemochromogen-like spectrum. Upon exposure of the reduced pyridine-hemoglobin solution to air the spectrum of the solution is immediately changed to that of an oxidized hemochromogen. The latter spectrum is also obtained on the addition of sufficient pyridine to oxyhemoglobin; but in this case no precipitate forms. The addition of KCN to the solution oxidized in the air gives rise to a spectrum indistinguishable from that of cyanhemoglobin prepared from methemoglobin.

While the spectra of reduced and oxidized globin hemochromogen are in general similar to those obtained by adding pyridine to hemoglobin, we have not succeeded in producing either the CO derivative of the reduced globin hemochromogen or the cyanhemoglobin-like derivative of the oxidized globin hemochromogen. Oxidized globin hemochromogen on standing exposed to air undergoes a further change, yielding a spectrum very similar to the ill defined spectrum obtained on adding 0.1 \( \text{N} \) \( \text{NaOH} \) to methemo-
globin. These findings suggest the interpretation that the reduced and oxidized derivatives produced by the addition of pyridine to hemoglobin are closer analogues of oxyhemoglobin and methemoglobin, respectively, than other hemochromogens thus far studied.

THE REMOVAL OF LACTIC ACID AFTER EXERCISE

BY H. T. EDWARDS, R. MARGARIA, AND D. B. DILL
(From the Fatigue Laboratory, Morgan Hall, Harvard University, Boston)

Blood lactic acid in recovery decreases according to a formula of the type log y = a - bt, in which y represents excess lactic acid and t the time. In most of our experiments in which the subject ran to exhaustion calculated values for lactic acid agreed with observed values within 2 or 3 per cent. A well trained athlete, running at 11.6 miles per hour on a 2.5 per cent grade, continued for $5\frac{2}{3}$ minutes, starting in a normal state, for $6\frac{1}{2}$ minutes after bicarbonate ingestion, for $7\frac{1}{2}$ minutes while breathing 40 per cent oxygen, and for $2\frac{2}{3}$ minutes while breathing 13.6 per cent oxygen. In such experiments of short duration, the observed and calculated values for lactic acid do not agree during the first stage of recovery; i.e., during the first 5 or 10 minutes of recovery. The duration of this first stage was found to be roughly inversely proportional to the length of the experiment.

Continuing our experiments of last year, in which samples of blood were withdrawn from the arm vein, femoral vein, and artery during recovery, we find that the divergence in lactic acid content of such specimens of blood decreases as the period of exercise lengthens. This suggests that the divergence is due principally to the idle tissues acting as a sink. The rôle of the liver in removing lactic acid appears to be of secondary importance.

THE REMOVAL OF IODOACETATE INHIBITION OF YEAST FERMENTATION

BY ELLEN EHRENFEST
(From the Laboratory of Biological Chemistry, Washington University School of Medicine, St. Louis)

The differential inhibition of iodoacetate on the fermentation and oxidation of glucose by yeast is thought by Lundsgaard\(^{30}\) to be

\(^{30}\) Lundsgaard, E., Biochem. Z., 250, 61 (1932).
the means of separating the two processes. Due to the presence of alcohol in the suspensions used by him we disagree with his interpretation for the following reasons.

Both fermentation and oxidation of glucose by a 0.6 per cent yeast suspension are inhibited by 2½ hours previous treatment with 1:90,000 iodoacetate. Alcohol added to such a suspension is readily oxidized. Although the inhibition of fermentation is maintained indefinitely under anaerobic conditions with such concentrations of iodoacetate, a period of oxidation of alcohol of 3 hours restores 50 per cent of the normal fermentation, and 5 hours, the normal rate. The apparent earlier recovery of oxidation of glucose can be explained by assuming that within the yeast cell the inhibitory action of the poison has been removed, and, if it could be measured, fermentation is also going on. Indeed, if transferred to nitrogen, such yeast shows a short period of fermentation. Oxidation of lactic acid is efficient in removing the inhibitory action. In the light of these experiments it is impossible to separate fermentation from oxidation by the iodoacetate procedure.

THE RELATION OF IRON AND COPPER TO THE RETICULOCEYTE RESPONSE IN ANEMIC RATS

By C. A. ELVEHJEM and M. O. SCHULTZE
(From the Department of Agricultural Chemistry, University of Wisconsin, Madison)

A study has been made of the daily changes in the hemoglobin, erythrocyte, and reticulocyte content of the blood of anemic rats when the milk diet was supplemented with iron alone, copper alone, and iron together with copper. The reticulocyte counts were made by the Friedlander and Wiedemer method and reported as number of reticulocytes per c.mm.

The addition of iron alone caused no significant change in the amount of hemoglobin, or the number of erythrocytes and reticulocytes. When copper was fed alone, no change was noted in the hemoglobin or erythrocytes, but there was a gradual increase in the number of reticulocytes. The maximum level of 1.2 millions per c.mm. was reached at about 8 days after the copper therapy was started. This high level often persisted for 8 to 10 days, with a gradual falling off thereafter.
The addition of iron and copper caused a very rapid reticulocyte response which reached a maximum of 1.6 to 1.8 millions per c.mm. 5 days after the iron and copper addition, and was followed by an almost equally rapid decrease to values approximately normal. The hemoglobin and erythrocyte count made a steady and rather rapid increase during the same period.

These results demonstrate that a typical reticulocyte response is obtained only when sufficient quantities of both iron and copper are supplied. The active material is probably a product formed by the interaction of the two elements in the animal body. Daily intraperitoneal injections of dilute hemoglobin solutions caused a decided and prolonged reticulocyte response, with only a slight increase in the hemoglobin and erythrocytes. The actual stimulation of reticulocyte formation may therefore be dependent upon the presence in the blood of hemoglobin or a closely related compound.

THE IRON AND COPPER CONTENT OF EGG YOLK

By Statie E. Erikson, Ruth E. Boyden, J. Holmes Martin, and W. M. Insko, Jr.

(From the Department of Home Economics, University of Kentucky, Lexington)

Determinations were made on the iron and copper content of yolks of eggs produced by hens given the same kind of all-mash ration, the differences in treatment being the administration of cod liver oil, sunshine, and open blue-grass range. Analyses for iron were made on 421 egg yolks and for copper on 212 egg yolks. The hens were kept in three pairs of pens. The hens of the first pair were confined without sunshine. Those of the second pair were confined with sunshine. Those of the third pair were allowed open blue-grass range. One pen of each pair had 2 per cent of cod liver oil added to the all-mash ration.

The results showed that the lowest amounts of both iron and copper were found in egg yolks produced by hens kept in confinement without cod liver oil, sunshine, or blue-grass. The administration of cod liver oil increased the amount of copper, sunshine increased it to about the same extent, and the effect of the two together was also about the same as that of either one alone.
The blue-grass range without cod liver oil showed a greater increase in the amount of copper in the egg yolk than sunshine alone, cod liver oil alone, or sunshine plus cod liver oil, while blue-grass plus sunshine plus cod liver oil showed the greatest increase of all.

The administration of cod liver oil to the hens had the effect of increasing the amount of iron in egg yolk, in all cases, but it was less effective in doing so when given with sunshine or with sunshine plus blue-grass. The highest value for iron was found in the egg yolks produced by hens given cod liver oil but deprived of sunshine and blue-grass.

FURTHER OBSERVATIONS ON THE CHEMISTRY OF INSULIN

BY E. A. EVANS, JR., AND E. D. SCHOCK

(From the Laboratory for Endocrine Research, the Johns Hopkins University, School of Medicine, Baltimore)

Previous work has indicated that the physiological action of insulin is dependent upon the presence of free amino groups and disulfide linkages in the insulin molecule. Both the Folin and Sullivan methods for cystine give definitely lower values than that required by the total sulfur. Whether this discrepancy is due to the presence of an unknown sulfur-containing constituent, or whether it is due to the partial destruction of cystine, made extremely labile by its mode of linkage, is problematical. It has been found, however, that under the very mildest conditions of alkaline inactivation the cystine shows a correspondingly small and definite decrease. This, in conjunction with the results obtained by a study of the behavior of insulin cystine under various other conditions, is in close agreement with previous findings, and indicates a relationship between physiological action and a portion of the cystine content of insulin. Evidence has also been obtained that a certain percentage of the free amino nitrogen of insulin is derived from the free amino groups of the cystine molecule. A study has also been made of the fraction containing non-amino nitrogen found among the hydrolysis products of insulin. In addition, the sensitivity of insulin to oxidizing and reducing agents has been further demonstrated by the finding that the hormone is inactivated by quinone and hydroquinone.
STUDIES ON THE DETERMINATION OF ALUMINUM IN ANIMAL TISSUES

BY DONALD F. EVELETH AND VICTOR C. MYERS
(From the Department of Biochemistry, School of Medicine, Western Reserve University, Cleveland)

A number of colorimetric procedures for the estimation of aluminum in animal tissues have been compared after both wet and dry ashing, and after separating the aluminum from other metals with the aid of three reagents, cupferron, thiocyanate, and sodium hydroxide. Aurin, alizarin, and 8-hydroxyquinoline have been used as the special reagents in the quantitative work, but aurin was found to be the most satisfactory, especially in the presence of minute amounts of aluminum. Although both aurin and alizarin gave identical figures on pure solutions, the results obtained on tissues were considerably higher when the alizarin method was employed. Data were obtained which indicate that the higher results obtained with alizarin were due to the presence of interfering substances which react with alizarin but not with aurin.

Analyses made on the tissue of the normal dog indicate that the values reported by Underhill and Peterman\textsuperscript{31} with the use of alizarin are too high. Aluminum administered to dogs intravenously appears to be widely distributed in the tissues, although it is stored primarily in the liver and spleen, and also in the kidney. Some of the injected aluminum promptly appears in the bile and urine, but a considerable amount of the aluminum is retained in the tissues of the dog for a long period of time. These data suggest that normally very little aluminum gets into the tissue from the alimentary canal, since normally it is present only in traces, but when aluminum does get into the tissues, \textit{e.g.} by the intravenous route, it is very slowly eliminated.

REDUCTION BY OLIGOSACCHARIDES

BY MARK R. EVERETT AND BEATRICE G. EDWARDS
(From the Department of Biochemistry and Pharmacology, University of Oklahoma Medical School, Oklahoma City)

Glucose reducing equivalents obtained by nitrophenolic and cupric reagents show an increasing divergence with increasing

\textsuperscript{31} Underhill, F. P., and Peterman, F. I., \textit{Am. J. Physiol.}, 90, 15 (1929).
molecular weight of sugars. The ratio, glucose equivalent by
Sumner's method to glucose equivalent by the Folin-Wu method,
averages 1.35 for eight monosaccharides (exclusive of methyl-
pentoses which have a higher ratio) and 1.55 for four disaccha-
rides. (See also Greenwald, Samet, Gross.32)

For higher oligosaccharides we find the following ratios: 2.35 for
amylotriose prepared by Pringsheim's method,43 4.3 to 5.0 for the
reducing substances in C.P. dextrins, 4.5 for inulin (by Kiliani's
method), and 2.7 to 5.3 for soluble starches, the latter increasing
with the blue iodine coloration. Similar high ratios are found for
the Benedict-Osterberg picrate method to Benedict's alanine
cupric method, etc. These effects are due to decreased reduction
of the cupric reagents by higher oligosaccharides and vary quanti-
tatively with different cupric reagents. The Hagedorn-Jensen
ferricyanide method gives results more like those obtained by the
nitrophenolic methods.

The absolute amount of reduction by different samples of soluble
starch, dextrin, and inulin is variable, but the high ratios are con-
stant, indicating the presence in these preparations of high molecu-
lar reducing impurities similar to "limit dextrin," amylose octadex-
trin, etc. Adding pure non-reducing polysaccharides to glucose,
maltose, etc., does not alter the original low ratios of the latter.
Early during acid hydrolysis of pure polysaccharides the expected
high ratios appear.

FURTHER EXPERIMENTS ON THE FAT METABOLISM HORMONE
OBTAINED FROM NORMAL URINE

By CASIMIR FUNK
(From the Casa Biochemica, Rueil-Malmaison, France)

It has been reported from this laboratory34 that we were able to
confirm the work of Anselmino and Hoffmann35 about the existence
of the fat metabolism hormone, with the important addition that

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34 Funk, C., and Zefirow, P. P., Sunti comunicaz. sc., XIV cong. internaz.
fsiol., Rome (1932).
the hormone was found in every normal urine, of man and animals, so far investigated.

The new hormone is best obtained from fresh urine by using the benzoic acid adsorption method described elsewhere. The precipitate formed is immediately filtered on a Buchner filter, dissolved in alcohol, and the residue washed with alcohol and weighed. The residue still wet (between 2.5 and 4.5 gm.) is triturated with a normal ammonia solution, 24 cc. per 3.6 gm. of the material being used. Using lesser or larger quantities of ammonia results in losses of yield. This crude extract when injected into rats appears to be very toxic, with a potency of over 100 mg. of total acetone formed after 2 hours per 100 cc. of urine, instead of 5 to 6 mg. as found in the controls. The toxicity can be eliminated by adding to the first ammoniacal extract acetone up to 60 per cent. After centrifugation of the large, in most cases inactive, precipitate, the active material is recovered from the supernatant fluid by acidification and addition of more acetone. The precipitate obtained is colorless, but yields when injected only 20 to 40 mg. of acetone per 100 cc. of urine. Whereas the crude ammoniacal extract possesses a marked diuretic action and at the same time increases the size of the male organs, the purified extract exhibits no diuretic effect, while the influence on the male organs appears very much decreased.

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<td>Weight and food utilisation</td>
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<td>Initial weight</td>
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<td>High fat; injected . . . . . . 121</td>
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To study the physiological action the purified preparation was injected daily (in doses corresponding to 30 to 60 mg. of acetone production in 2 hours) into rats on a practically fat-free diet, on the one hand, and on a diet containing 40 to 73 per cent fat on the other hand. After 64 days the animals were killed and analyzed in toto. A summary of the results is shown in Table I.

The injected animals showed better gains in weight and this was especially marked in the group fed the high fat diet; upon discontinuing the injections a marked drop in weight usually occurred. Furthermore, the injected animals showed a better utilization of food, a diminished fat, and increased water content. The decreased urinary flow on high fat diets has recently been described elsewhere.37

Next the response to a single injection on these two diets was investigated and it was found that on the high fat diet the response is less (81 mg. on the average) than on the fat-free diet (108 mg.). Changing the diet from a fat-free to a high fat diet decreases the amount of material adsorbed by the benzoic acid and causes a disturbance of the hormone excretion in urine; but no definite conclusions can be reached at present. The oxalated blood of the injected animals shows definite evidence of a continued chemical reaction on standing, as if the hormone action were followed by an enzymic reaction. This phase of the work is now under investigation.

For the demonstration and determination of the hormone in urine the following short procedure is recommended. 5 to 20 cc. of urine are placed in a centrifuge tube, treated with 0.6 gm. of benzoic acid in alcohol, centrifuged, and the residue washed with alcohol. The residue is weighed, extracted with ammonia, in proportions given above, again centrifuged, and the supernatant liquid neutralized and injected. The response obtained is often as much as 50 mg. of acetone per 5 cc. of urine.

In order to test whether the hormone acts directly or by way of the thyroid (thyrotropic hormone ?), a number of thyroidec- tomized rats were injected. The response was insignificantly lower than in normal controls.

FURTHER STUDIES OF ANTERIOR PITUITARY EXTRACTS

BY OLIVER HENRY GAEBLER

(From the Department of Laboratories, Henry Ford Hospital, Detroit)

In previously reported experiments the globulin fraction of beef anterior pituitary glands, which stimulates growth, was injected into bitches for 1 or 2 days. The effects on the nitrogen balance, water intake and output, body weight, and energy metabolism were then observed daily until the previous equilibrium was reestablished. In such experiments a very large increase in the nitrogen balance occurs during the week after injection, and is frequently followed by nitrogen loss during the 2nd and 3rd weeks. There are large increases in water intake and urine volume and sudden but transient gains in weight. A remarkable increase in metabolism appears within 24 hours and gradually subsides in 4 to 8 days after single injections. Simultaneously, the basal respiratory quotients fall, owing to increased oxidation of fat, and return to their initial level.

Studies of the same type have been continued in order to determine (1) whether any one of the three characteristic responses (altered nitrogen balance, water balance, and energy metabolism) is due to a substance in the injected extract to which an animal establishes immunity after repeated injections, (2) whether the three reactions are also obtained with pituitary extracts that have little or no growth-promoting action, and (3) whether the results are the same in normal and thyroidectomized dogs.

In one animal all three of the characteristic responses to the injection of extract suddenly disappeared, but after an interval of 2 months all were again observed in the same animal after an injection of another portion of the same lot of extract.

The anterior pituitary-like substance from urine from a pregnant case caused nitrogen loss instead of storage, and a very transient elevation of metabolism. An unpurified alkaline extract of the whole pituitary gland of sheep, which does not stimulate growth, caused nitrogen loss, but brought about an elevation of metabolism as striking and prolonged as that produced by the growth-promoting fraction of beef anterior lobes. The last mentioned preparation produces all of its characteristic effects in thyroparathyroidectomized bitches kept in good condition by

means of parathormone. The data are not as yet extensive enough to show whether the effect of this extract on metabolism is somewhat diminished in intensity after thyroidectomy, but the duration of the increase in metabolism appears to be unchanged, as are also the parallel changes in the respiratory quotient.

THE PROBLEM OF HEN-FEATHERING IN SEBRIGHT COCKS

BY T. F. GALLAGHER, L. V. DOMM, AND FRED C. KOCH
(From the Department of Physiological Chemistry and Pharmacology and the Whitman Laboratory, the University of Chicago, Chicago)

The hen-feathering of the Sebright cock has been for some time the subject of much speculation and experimentation by sex physiologists. Morgan first ascribed the hen-feathering to a female secretion from the testes. Pédard claimed the Sebright testis exerted a feminizing action on the plumage of normally cock-feathered races as well as on the Sebright. Roxas from an extensive series of cross-transplantations concluded that the hen-feathering in Sebrights was a genetic character and a normal reaction of the soma to the testicular hormone. Benoit has recently reopened the question and postulates a plumage-inhibiting hormone which is secreted by the testes of all races but in the Sebright is present in such large amounts that plumage is transformed in the female direction.

The solution to this problem is provided by the administration of highly purified testicular extract. This extract has no effect upon the plumage of cock-feathered races as has been shown before and is amply confirmed by the controls in the present series. The extract in all cases produced perfectly typical hen-feathering in Sebright capons. Our results completely confirm the conclusions of Roxas and invalidate Benoit’s sole objection to his experiment.

EFFECT OF ALKALI ON THE COMB GROWTH-STIMULATING MALE HORMONE

BY T. F. GALLAGHER AND FRED C. KOCH
(From the Department of Physiological Chemistry and Pharmacology, the University of Chicago, Chicago)

Male hormone preparations obtained from bull testicle tissue and from human urine respectively were carefully assayed by the
comb growth method. These preparations behave differently when treated with 5 per cent alcoholic potassium hydroxide at the boiling point. Such treatment of the tissue extract product results in the rapid loss of the comb growth activity. However, the urinary product retains its activity under like treatment. Control experiments show that the loss in the case of the testis tissue product is not due to mechanical loss in the recovery by immiscible solvents nor to the production of toxic antagonistic substances. Although the biological activities of the tissue and urinary products appear to be identical, the resistance toward the action of alkalies is very different. Hence there must be a chemical difference.

STUDIES ON THE TESTICULAR HORMONE FROM HUMAN URINE

BY T. F. GALLAGHER AND FRED C. KOCH
(From the Department of Physiological Chemistry and Pharmacology, the University of Chicago, Chicago)

The activity is first extracted from acidified urine by benzene. After evaporation of the benzene and re-solution in ether, considerable inert material is removed by shaking with 10 per cent sodium hydroxide solution. The product thus obtained represents approximately 1 mg. per unit. Distillation of this product at pressures of 0.01 to 0.1 \( \mu \) and temperatures up to 150° removes approximately 40 per cent by weight of inactive material, with almost complete recovery of the activity in the undistilled residue. Treatment of this residue with various combinations of immiscible solvents results in approximately 70 to 75 per cent of recovery of the activity. The potency of the final product is of the order of 0.15 to 0.2 mg. per bird unit. Redistillation of this product between 150-200° at 0.01 to 0.1 \( \mu \) pressure now yields a distillate weighing 30 to 70 per cent of the product distilled. From the distillate a mixture of oximes can be obtained.

TECHNIQUE AND ACCURACY OF THE TESTICULAR HORMONE ASSAY

BY T. F. GALLAGHER AND FRED C. KOCH
(From the Department of Physiological Chemistry and Pharmacology, the University of Chicago, Chicago)

The assay for testicular hormone by the capon comb growth method has been extended and improved. Statistical methods
demonstrate a correlation between comb size and the final growth obtained. Body weight of the animal has little significance. The 2 day and 5 day periods are compared and characteristic curves presented for each. The accuracy of both methods is compared and a bird unit is redefined in terms of a standard to which the necessary corrections are applied.

THE DIFFERENTIAL METABOLISM OF THE AMINO ACIDS

BY LOUIS P. GERBER, ERNST K. NIELSEN, AND RALPH C. CORLEY

(From the Laboratory of Biochemistry, Department of Chemistry, Purdue University, Lafayette)

There has been in progress and in project for a number of years an investigation of the treatment accorded by the tissues to the essential as contrasted with the presumably non-essential amino acids. Will there be detectable differences in the manner of disposal of these moieties under conditions of stress, specifically when there exists a hunger for nitrogen?

In an earlier attack,39 the disposal of several amino acids was followed by analyses of blood and urine after oral and intravenous administration to fasting rabbits, and to dogs on rations low in protein. The results were suggestive but it has seemed desirable to employ more rigorous conditions of experiment before attempting any generalizations.

In the present study, we have determined the effect of a number of amino acids on the nitrogen and sulfur balances and on the urinary nitrogen to sulfur ratios of dogs maintained on nitrogen-low synthetic rations furnishing about 10 to 15 mg. of nitrogen and 80 to 90 calories per kilo of body weight. There has been found a distinct retention of a portion of the nitrogen of leucine, arginine, lysine, and histidine. With tyrosine there has been but questionable evidence of retention. While the results are somewhat more variable with alanine, glycine, and glutamic acid, those recorded thus far give no evidence of retention of nitrogen, and indeed in several experiments there was a loss of extra nitrogen.

The figures for sulfur excretion and the nitrogen to sulfur ratios offer no definite evidence of any influence of the administered amino acids on the metabolism of sulfur or the tissues containing sulfur.

THE EFFECT OF ELECTROLYTES ON THE OXYGEN-HEMOGLOBIN EQUILIBRIUM

BY ARDA ALDEN GREEN AND J. H. TALBOTT
(From the Fatigue Laboratory, Morgan Hall, Harvard University, Boston)

The oxygen dissociation curve of the hemoglobin of the horse had been studied at pH 7.4 and 38° in solutions of varying concentration of sodium chloride. The pH of the equilibrated solutions was determined on the glass electrode.

Both the shape and position of the curve depend upon the salt concentration. The curves are moved symmetrically to the right with increasing salt concentrations in concentrations of 0.1 M to 2 M NaCl. In lower concentrations of electrolyte the shape of the curve also changes.

The effect may be most easily described if the per cent saturation is plotted against the logarithm of the oxygen tension and the resulting curve is assumed to be the sum of two curves in which the hemoglobin combines with 1 or with 3 molecules of oxygen at a time.

THE SYNTHESIS OF HIPPURIC ACID IN RATS

BY WENDELL H. GRIFFITH
(From the Laboratory of Biological Chemistry, St. Louis University School of Medicine, St. Louis)

Since earlier investigations of the effect of the reaction of the diet on the detoxication of benzoic acid in animals have not given concordant results, a study has been made of the influence of the reaction of the diet on the synthesis of hippuric acid in rats. A modification of the method which has been described previously was used.

Survival, increase in weight, and excretion of hippuric acid were determined in groups of young rats fed an adequate food mixture plus a toxic quantity of benzoate. Sodium benzoate was slightly less toxic than an equivalent amount of benzoic


acid. The addition of NaHCO₃ to sodium benzoate further decreased the toxicity of the diet. The addition of 1 equivalent of NaHSO₄ or NH₄Cl to benzoic acid increased the toxicity of the diet. In these experiments, an increase in the base of the diet exerted a favorable influence upon the detoxication of benzoate.

The toxicity of the benzoate was markedly decreased either by glycolic acid or by glycolic acid plus 1 equivalent of NaHCO₃. The effect of the administration of glycolic acid in these feeding experiments was the same as that resulting from the administration of glycine.

THE DETERMINATION OF IRON IN BIOLOGICAL MATERIAL

BY R. F. HANZAL

(From the Institute of Pathology and the Department of Biochemistry, School of Medicine, Western Reserve University, Cleveland)

A study has been made of the reaction between ionic iron and thioglycolic acid with the intention of applying this reagent to the colorimetric determination of iron in biological material. It has been found that in low concentrations of iron, with a relative excess of the thioglycolic acid, the purple color developed is proportional to the concentration of iron. It has also been found that this reagent is specific for both ferrous and ferric iron in the range of iron concentration used in a quantitative method. The effect of interfering substances such as pyrophosphates is less pronounced than in the case of the reaction between ferric iron and thiocyanates. In order to eliminate the effect of interfering substances and also to concentrate iron in solution, a study was made of the quantitative precipitation of iron by means of cupferron, the ammonium salt of nitrosophenylhydroxylamine.

The method applicable to all biological material essentially consists of (1) dry or wet ashing of the material with acid solution of the dry ash, (2) precipitation of iron by means of cupferron in the acid digest, (3) digestion of the ferric-cupferron precipitate, (4) development of color with thioglycolic acid in slightly ammoniacal solution and matching against an appropriate standard.
THE MALE HORMONE
VI. FURTHER EXPERIMENTS ON THE EFFECT OF THE MALE HORMONE AND THE ANTERIOR PITUITARY

BY BENJAMIN HARROW AND BARNET NAIMAN
(From the Department of Chemistry, College of the City of New York, New York)

AND CASIMIR FUNK
(From the Casa Biochemica, Rueil-Malmaison, France)

We have continued and extended the work already reported* and which has found confirmation in the paper by Graber and Cowles. In these preliminary experiments we have, however, replaced the urine of pregnant women by a commercial preparation of the anterior pituitary hormone.

For each of the following experiments six rats (four males and two females) were used, and the injections were carried on for 5 days. The seminal vesicles and the testes in the male and the

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* 1 cc. of the Squibb's original solution, follutein, is equivalent to 1250 rat units. We diluted 1 cc. to 312 cc. with distilled water, so that 1 cc. of the diluted material corresponded to 4 rat units, and the 0.5 cc. which we used for injection corresponded approximately to 2 rat units. The Squibb's follutein was kindly supplied to us by Dr. J. A. Morrell of E. R. Squibb and Sons.

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tubes and the ovaries in the female were weighed and their percentages in terms of the weight of the rat at the time of death were calculated. The averages are presented in Table I.44

The results fully confirm the accelerating effect on seminal vesicle growth when both the male hormone and the anterior pituitary are injected. The effect on the tubes in the female, if somewhat less striking, is nevertheless evident.

We are engaged in fractionating experiments on urine from normal and pregnant cases to determine whether fractions other than the so called “anterior pituitary fraction” have any effect in conjunction with the male hormone. These fractions have already been prepared by one of us (C. F.).

PANCREATIC ENZYMES IN PERNICIOUS ANEMIA

By O. M. HELMER, PAUL J. FOUTS, AND L. G. ZERFAS
(From the Indianapolis City Hospital, Indianapolis)

The fasting gastric contents and the duodenal juice after acid stimulation were studied in five normal controls and in twenty-two patients with pernicious anemia. The amylase, lipase, trypsin, and trypsinogen were determined. Though there was considerable variation in the values found, pancreatic enzymes were always present in the duodenal juice. Patients with pernicious anemia who had lowered amounts, especially of trypsin and trypsinogen, had moderate to advanced central nervous system involvement. The tryptic activity of the duodenal juice was considerably enhanced by activation with enterokinase. The gastric contents of fasting subjects were of no value in determining the external secretory activity of the pancreas.

AUTOLYSIS AS A METHOD FOR THE PREPARATION OF TERAPEUTICALLY ACTIVE SUBSTANCES IN TISSUES

By WILLIAM F. HERRON and WILLIAM S. McELLROY
(From the Department of Physiological Chemistry, School of Medicine, University of Pittsburgh, Pittsburgh)

We have observed that autolysis increases the therapeutic activity of liver in the treatment of pernicious anemia45 and certain

44 We are indebted to Dr. Perla, of the Montefiore Hospital, New York, for the histological work connected with these experiments.
types of secondary anemia. In pernicious anemia the increase in potency applied to all of the characteristic features of a favorable response to liver therapy, as increase in red blood cells, white blood cells, reticulocytes, and platelets, with reduction in coagulation time and improvement in symptoms, especially those referable to the nervous system. With reference to platelet formation a striking response to autolyzed liver was observed in the two cases of purpura hæmorrhagica treated. To explain the increased activity after autolysis, we have considered two obvious possibilities: (1) by producing a more or less complete physical disintegration of the tissue, autolysis favors more complete extraction of the substance or substances already present in the tissue in the active form; (2) that an actual increase in the active substance or substances occurs. We are inclined to the view that increased activity is due to an actual increase in the active substance, because in a case of typical pernicious anemia a favorable response was obtained from autolyzed beef muscle. Application of the method to the preparation of other tissue extracts is being investigated.

THE GASOMETRIC METHOD FOR THE ESTIMATION OF CYSTEINE AND CYSTINE

BY W. C. HESS

(From the Chemo-Medical Research Institute, Georgetown University, Washington)

Baernstein has described a method for the determination of cysteine and cystine based upon the reaction of cysteine and cystine, after reduction, with iodine in strongly acid solution. The excess iodine is estimated in the Van Slyke apparatus by allowing it to react with alkaline hydrazine and measuring the liberated nitrogen. The possible errors in this method will be shown to be due to the reaction of iodine with certain hydrolytic decomposition products which may not, necessarily, be due to cysteine.

EFFECT OF ULTRA-VIOLET IRRADIATION ON THE VITAMIN B COMPLEX

BY ALBERT G. HOGAN AND LUTHER R. RICHARDSON

(From the Departments of Animal Husbandry and Agricultural Chemistry, University of Missouri, Columbia)

Some of the precautions adopted in our original procedure have proved unnecessary. The rats are prepared for use by placing them, and their mothers, on the experimental ration at the age of 15 days. They are transferred to metal screens at the same time, and are weaned when 21 days old. The experimental ration consists of casein, leached with acidified water 20, commercial sucrose 71, cellulose 3, a mineral mixture 4, and cod liver oil 2. A mixture of equal parts of tikitiki and liver extract is used as a source of water-soluble vitamins. This is irradiated for 15 hours in a thin layer, with constant stirring. The depletion period is omitted.

Dermatitis has not appeared when corn-starch was substituted for sucrose, and the gains in weight were normal. When 13 parts of butter, lard, or Crisco were substituted for an equal amount of sucrose, dermatitis did not always develop, and the survival periods were appreciably longer.

The lesions were healed by untreated tikitiki, but were succeeded by others of a different external appearance. Dermatitis did not develop when irradiated vitamin B carriers were supplemented with autoclaved tikitiki, but the mortality rate was not reduced. The untreated liver extract afforded partial protection but this was lost on heating in an autoclave. Fullers' earth activated with yeast healed the lesions promptly. Vitamin B supplements irradiated through ordinary window-glass showed little or no loss in activity.

CALCIUM AND PHOSPHORUS STORAGE IN GROWING CHILDREN

BY HELEN A. HUNSCHER, FRANCES COPE, ALICE NOLL, AND ICIE G. MACY

(From the Research Laboratory of the Children's Fund of Michigan, Detroit)

Twelve children ranging from 3 to 10 years of age have been maintained on acid-base mineral balance studies under identical experimental conditions during two to twenty consecutive 5 day
periods. The diet was kept constant in mineral content at all times except for four children who received an addition of 600 gm. of whole milk, during four to eight consecutive periods. Only the calcium and phosphorus retentions on 58 balance studies including twenty, fourteen, thirteen, and eleven consecutive periods for four children respectively are herein reported. When the basal diet contained 1 gm. of calcium, the average retentions were 0.26, 0.31, 0.30, and 0.48 gm. per day, but when the calcium intake of the first three children was increased to 1.9 gm. per day the average retentions were increased to 0.55, 0.64, and 0.67 respectively. In a like manner the basal diet containing 1.3 gm. of phosphorus gave average retentions of 0.36, 0.40, 0.35, and 0.43 gm. respectively for the four children, whereas an increased intake to 2 gm. for the first three children resulted in the storage of 0.6, 0.58, and 0.52 gm. respectively. There was an appreciable variation of retention from period to period on each level of calcium and phosphorus intake. Furthermore, there was a distinct difference not only in the amounts retained on the two levels of calcium and phosphorus in the food intake but also in the magnitude of retention in the individual children.

A NEW METHOD OF DETERMINING THE FRAGILITY OF BLOOD PLATELETS

BY DON D. IRISH

(From the Laboratory of Biochemistry, University of Cincinnati, Cincinnati)

Blood platelets may be caused to disintegrate by a hypotonic solution of sodium chloride. The platelets to be tested are washed free from plasma and suspended in 0.85 per cent sodium chloride. A measured quantity of suspension is added to a series of tubes containing varying concentrations of sodium chloride and a small quantity of calcium chloride. After allowing a definite time for disintegration, a portion of platelet-free plasma or a solution of blood fibrinogen is added, thus completing a clotting system. In the tubes where disintegration has occurred clotting will occur. The fragility is expressed as the concentration of sodium chloride just sufficiently hypotonic to cause disintegration of enough platelets to facilitate rapid clotting.
THE CHEMICAL STUDIES OF TOAD POISONS

BY H. JENSEN, K. K. CHEN, AND A. L. CHEN

(From the Laboratory for Endocrine Research, the Johns Hopkins University, School of Medicine, Baltimore, and The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis)

From the secretion of different species of toads, various principles have been isolated. These may be classified in three main groups: (1) basic constituents ((a) epinephrine, (b) bufotenines), (2) bufagins, and (3) bufotoxins.

The bufotenines are obtained as flavianate salts; some of these compounds raise the blood pressure and stimulate smooth muscle. Evidence has been obtained that they contain the indole ring structure. They seem, however, not to be related to the betaine of tryptophane, as has been suggested by Wieland and coworkers.

The bufagins are unsaturated neutral compounds containing a lactone group and several hydroxyl groups, one of which is linked to a fatty acid, e.g. formic acid or acetic acid. They have a physiological action on the heart similar to that of the digitalis glucosides.

The bufotoxins are composed of the corresponding bufagins linked to suberyl arginine. Their physiological action is similar to that of the bufagins.

There seems to exist a chemical relationship between some of the bufagins and the cardiac aglucones of the plant kingdom, as there is also a close pharmacological resemblance. The chemistry of marinobufotoxin, of marinobufagin, of cinobufagin, and of bufotalin chloride has been studied in more detail. The empirical formula for cinobufagin, obtained from Ch’an Su, is C_{26}H_{32}O_{6}, and for marinobufagin, obtained from Bufo marinus, C_{24}H_{32}O_{5}. By splitting off the acid radical which is attached to a hydroxy group (acetic acid from cinobufagin and formic acid from marinobufagin), one obtains compounds which are C_{23} derivatives, as are the aglucones of the plant glucosides.

From the secretion of Bufo arenarum a sulfur-containing compound was obtained which is identical with bufotothionine, isolated by Wieland and coworkers from the secretion of the Japanese toad.
DIGESTION STUDIES IN VITRO. SOME PARTIAL CLEAVAGE PRODUCTS FROM A PEPTIC DIGEST OF CASEIN

By D. BREESE JONES and CHARLES E. F. GERSDORFF
(From the Protein and Nutrition Division, Bureau of Chemistry and Soils, United States Department of Agriculture, Washington)

In connection with preliminary experiments on the rate of liberation of certain amino acids from proteins when digested in vitro, an examination was made of some partial cleavage products of casein after 1 hour's digestion with pepsin. Two main fractions were separated. Fraction A, a flocculent, gelatinous material suspended in the digest, amounted to about 29 per cent of the original casein. A small fraction, Fraction C, was also obtained by adjusting the filtrate from Fraction A to pH 6. It represented only about 1.3 per cent of the original casein. Fraction C represented the soluble portion remaining in the digest after removal of Fraction B.

Analyses of the two main fractions showed striking differences in composition. Fraction A contained nearly all the phosphorus of the original casein, while Fraction C had practically all of the cystine. Fraction C contained more than 4 times as much tryptophane as was found in Fraction A, and nearly 2 times as much tyrosine. It also contained most of the basic nitrogen.

CREATININE DETERMINATION WITH THE PULFRICH PHOTOMETER

By BEATRICE KASSELL
(From the Department of Chemistry, New York State Psychiatric Institute and Hospital, New York)

The quantitative absorption spectra of picric acid, of sodium picrate, and of the red tautomer of creatinine picrate (formed in the Jaffe reaction) were determined with the aid of the Pulfrich photometer. Thus the conditions were established for a spectrophotometric determination of creatinine. Filters S-50 and S-53 are used: No. S-53 for higher concentrations of creatine or creatinine (urine and muscle), No. S-50 for lower concentrations (blood and small amounts of muscle).

Although Beer's law apparently holds true over the range in which determinations can be carried out, minor deviations occur,
owing to the fact that the filters are not strictly monochromatic. It is therefore necessary to prepare calibration tables. The extinction coefficient of a solution containing 1 mg. of creatinine, 20 cc. of 1.2 per cent picric acid, and 1.5 cc. of 10 per cent sodium hydroxide in 100 cc. is 0.433 with Filter S-53 and 1.159 with Filter S-50. (The absorption due to sodium picrate is excluded from the measurement by placing an alkaline picrate solution in front of the opposite aperture of the photometer.) Creatinine may be determined in quantities as small as 1 μg (0.2 μg with microequipment).

Spectrophotometric determinations of creatine in urine show that the colorimetric method gives results sometimes as much as 10 per cent too high, depending on the method used.

The advantages of the spectrophotometric determination of creatinine are manifold. The green colors of Filters S-50 and S-53 cause less eye fatigue and increase the accuracy and speed of reading. Interfering colors like that of impure picric acid and those formed in the autoclaving of urine are filtered out. Determinations with the Pulfrich photometer are carried out more rapidly than with the colorimeter, because it is no longer necessary to prepare standards, and the creatinine content of almost any solution may be ascertained directly from the calibration tables.

THE PHYSIOLOGICAL ACTION AND THE CHEMICAL NATURE OF THE ACTIVE PRINCIPLE IN THE SUPRARENAL GLAND ESSENTIAL TO LIFE

By Edward C. Kendall, Harold L. Mason, Bernard F. McKenzie, and C. S. Myers

(From the Division of Chemistry, The Mayo Foundation, Rochester, Minnesota)

A method for the separation of the active principle from the suprarenal gland essential to life consists of an extraction with acetone in the presence of sulfuric acid at pH about 3. In the absence of the acid the amount extracted with acetone is only a small percentage of the total amount present. After concentration of the acetone solution to a small volume, the epinephrine, hexuronic acid, lactic acid, and other substances are removed with lead acetate and sodium bicarbonate in the presence of methyl alcohol and acetone. From the fraction soluble in acetone the
last traces of epinephrine are removed with solid lead nitrate and anhydrous potassium carbonate.

If the administration of the hormone to a dog after double suprarenalectomy is stopped, the dog will not live more than from 4 to 7 days. Although the blood volume is low at time of death, this is only one of many changes which develops. It does not appear to be the primary cause of death.

The hormone appears to consist of three parts. The identity of these three parts and the chemical groups which are involved in the links between the three parts, together with the possible explanation of the chemical reactions involved and physiological significance of the compound, will be given.

REPRODUCTION AND LACTATION IN THE ABSENCE OF THE PARATHYROID GLANDS AND THEIR PROBABLE FUNCTION

By F. L. KOZELKA

(From the Department of Agricultural Chemistry, University of Wisconsin, Madison)

Thyroparathyroidectomized dogs can complete a life cycle including reproduction and rearing of the young provided a sufficient amount of vitamin D is fed to maintain a normal serum calcium and phosphorus level. Growing puppies required 26.6 Steenbock rat units, while gestating females required a progressively increasing amount up to the day of parturition, when they required 26,000 rat units.

Rachitic dogs with subnormal serum calcium and phosphorus level were found to be immune to 80 units of Collip's parathormone. Intravenous injection of CaH₄(PO₄)₂ or the addition of lactose to the diet for 5 weeks did not destroy the "immunity" even though the lactose increased calcium and phosphorus absorption. Feeding 26.2 rat units of vitamin D daily did not cause a response to the parathormone until at the end of the 3rd week, while irradiation for 1 hour daily caused a reaction to the parathormone immediately. Parathyroidectomized dogs did not respond to irradiation regardless of the length of time they were exposed to the light, indicating that the parathyroid hormone functions by liberating the vitamin D from the cutaneous and subcutaneous tissues and making it available to the animal.
THE METABOLISM OF CITRIC ACID

BY ADRIAN C. KUYPER AND H. A. MATTILL

(From the Laboratory of Biochemistry, State University of Iowa, Iowa City)

The metabolism of citric acid is closely related to the acid-base balance, as suggested by Ostberg, but also to metabolic processes in general. A short period of vigorous muscular exercise, perhaps because of the attendant production of lactic acid, causes a prompt decrease in the amount of citric acid excreted; an alkalosis produced by accelerated breathing quickly increases the amount. Immediately following a meal and after large doses of citric acid itself, there is an increased excretion in the urine.

A comparison between the small changes in blood citrate produced by administration of alkali to a rabbit and the rapid decrease in the blood citrate of a fasted rabbit indicates that the nutritive condition of the animal is the more important factor in determining the citrate content of the blood. When citrate is fed to a fasted rabbit, the concentration in the blood increases only slightly and for a brief period. Analyses of blood drawn from the arterial system and from the renal vein indicate that citrate is not made by the kidney. The opposite conclusion, which has been suggested by others, may have its basis in the interference of inorganic salts in the accurate determination of citrate by the Thunberg methylene blue method.

EFFECT OF STARTLE ON ELECTROCARDIOGRAM AND RESPIRATION FOLLOWING INJECTION OF ADRENALIN*

BY CARNEY LANDIS

(From the Department of Psychology, New York State Psychiatric Institute and Hospital, New York)

Electrocardiographic records (Lead II) and pneumographic tracings were taken on a group of normal individuals. At intervals during the first 30 minutes of the experiment the subject was stimulated by a yell, horn, or bell. Heart and respiratory tracings were made before and after each stimulation. Then 1.25 cc. of adrenalin were administered intramuscularly. Subsequent to the adrenalin, the same stimuli were employed at irregular inter-

* Presented before the American Physiological Society.
The records were analyzed particularly for the relationship existing between the point in the cardiac or respiratory cycle at which the stimulation took place and the appearance of any variety of cardiac or respiratory irregularity.

We found: (1) Electrocardiographic irregularities of a variety of forms occasionally occur in the first heart beat following stimulation. Some of these changes are due to the heart itself; others are technical artifacts. (2) Confirming the previous reports of Landis and Slight, Messerle, and Blatz on non-adrenalinized subjects, the time of stimulation with respect to cardiac or respiratory cycle was directly related to the production of "cardiogram irregularities." (3) Stimuli which occurred during the adrenalinized state were no more effective in producing changes in pulse rate than stimuli at other periods. (4) Of the stimuli which produced a galvanic skin response, there was no difference which could be attributed to the adrenalin. Factors in the control of the heart, which are illustrated by the electrocardiographic irregularities, as well as the relation between the temporal point of stimulation and the "cardiogram irregularities," are considered.

THE OXYGEN EQUIVALENTS OF THEELIN AND THEELOL AND OF SOME OF THEIR DERIVATIVES

BY LOUIS LEVIN, D. W. MACCORQUODALE, SIDNEY A. THAYER, AND EDWARD A. DOISY

(From the Laboratory of Biological Chemistry, St. Louis University School of Medicine, St. Louis)

Quantitative determinations of the oxygen-consuming power of theelin and theeol and of some derivatives of these compounds have been made in an effort to gain some insight concerning the constitution of these substances. The method used was that developed by Smith and Spoehr (1930), involving the oxidation of the organic substance by means of potassium permanganate in pyridine solution, the excess of the oxidizing agent being estimated by reduction with a standard oxalic acid solution and titration of the remaining oxalic acid with standard aqueous permanganate.

In the case of theelin and its derivatives well defined end-points were obtained, the reduction of the potassium permanganate practically ceasing after a few hours. In no case were the results
so satisfactory with theanol and its derivatives. With these com-
ounds there was a well defined slackening in the rate of reduction
of the permanganate but no point was reached at which the reac-
tion was obviously at an end. Permanganate continued to be
consumed at a slow and constant rate for many hours after the
initial rapid oxidation was over. This was, no doubt, due to
gradual oxidation of the primary oxidation products.

It was found that 30° was the most satisfactory temperature for
these oxidations. At this temperature the initial oxidation was
more rapid than at 25° and the change in the rate of oxidation was
more sharply defined.

In all cases methylation of the phenolic hydroxyl group resulted
in a decrease in the oxidation equivalent but this decrease was not
constant for the different compounds investigated. Thus the
methyl ether of theelin required 7 less atoms of oxygen per mole-
cule than theelin, whereas the difference between theanol and its
methyl ether was found to be 6 atoms.

FURTHER EVIDENCE CONCERNING THE MULTIPLE NATURE OF
THE VITAMIN B COMPLEX OF YEAST

BY ROBERT C. LEWIS AND MARION R. RYMER
(From the Department of Biochemistry, University of Colorado School of
Medicine, Denver)

Further experiments confirm the earlier reports47 from this
laboratory that the residue remaining after four extractions of
dried bakers' yeast with progressively stronger concentrations of
alcohol (50 to 95 per cent) contains a factor, or factors, distinct
from vitamins B and G and necessary for the growth of rats.
Although markedly subnormal growth occurred when yeast extract
(vitamins B and G) supplemented a Sherman and Spohn diet,48
the further addition of the yeast residue produced growth com-
parable to that obtained when whole yeast was used in place of
its various fractions. The active principle of the residue is stable
to acid autoclaving at 120° for 5 hours at pH 5.3, but is largely
destroyed by the same treatment at pH 9.4. Thus, the factor is
not as thermostable as previously supposed.

The residue was further extracted fourteen times with 0.1 per cent acetic acid according to the method of Hunt and Wilder. Rats placed on supplements of vitamins B and G plus both the acetic acid extract of the residue and the residue remaining after acetic acid extraction showed a growth superior to those receiving vitamins B and G plus only one of these fractions; i.e., either the acetic acid extract or the acetic acid-extracted residue. Doubling the amount of either fraction failed to induce a greater amount of growth. These results suggest that the growth principle present in the residue remaining after the alcoholic extraction of yeast may consist of two factors, neither of which is vitamin B or vitamin G and both of which are necessary for the normal nutrition of the rat.

THE EFFECT OF THE INGESTION OF TYROSINE ON THE BLOOD PHENOLS AND THE BLOOD URIC ACID AS DETERMINED BY THE METHODS OF FOLIN AND OF BENEDICT

By JOSEPH M. LOONEY

(From the Memorial Foundation for Neuro-Endocrine Research and the Worcester State Hospital Laboratories, Worcester)

1 gm. of tyrosine dissolved in 400 ml. of water was given to each of forty-eight patients suffering from schizophrenia, after specimens of blood and urine were taken during fasting. Samples of blood were taken after 3 hours and again after 6 hours and the urine was also collected during these intervals.

The blood specimens were analyzed for free and conjugated phenols by the method of Theis and Benedict, and for uric acid by the direct method of Benedict and also by the latest modification of the Folin method. Qualitative tests were made for tyrosine by a modification of the method of Folin and Marenzi.

It was found that the free phenols increased during the first 3 hours from 1.24 to 1.30 mg., and showed a maximum increase of 0.13 mg. when the highest value for each patient for the 6 hour period was taken. The odds that these changes were due to chance are 77:1 against it in the first instance, and 50,000:1 in the second.

During the same time the uric acid as measured by the Benedict method increased 0.16 mg., from 3.46 to 3.62 mg. This change must also be ascribed to the effect of the tyrosine ingestion, as the odds against the occurrence being due to chance are 100:1. The uric acid as measured by the Folin method at the same time remained constant at 2.98 mg., an increase of only 0.01 mg. being obtained.

A definite correlation was found between the variation of free phenol from 8.00 to 11.00 a.m. and that of the uric acid as determined by the Benedict method at the same time. The correlation coefficient was 0.32. This correlation tends to be masked somewhat by the tendency of the free and conjugated phenols to show an inverse change, having a correlation coefficient of −0.33. No correlation was noted between the variation of free phenols and that of uric acid as determined by Folin's method, the coefficient being 0.00.

The correlation coefficient between the uric acid determinations by the two methods was 0.77. This would be a very high coefficient for showing causal relationship, but is too low to predict the value of uric acid by one method when the other is known. It indicates that the two methods are not measuring absolutely identical substances, as Benedict's method is affected by changes in phenols as well as by changes in uric acid.

No association could be shown between the changes in blood phenols and the increases in the tyrosine of the blood. The excretion of phenol in the urine could not be correlated with the phenol content of the blood.

THE POSSIBLE MECHANISMS OF CONTRACTING AND PAYING THE OXYGEN DEBT AND THE RÔLE OF LACTIC ACID IN MUSCULAR CONTRACTION

By R. MARGARIA, H. T. EDWARDS, L. J. HENDERSON, AND D. B. DILL

(From the Fatigue Laboratory, Morgan Hall, Harvard University, Boston)

When muscular exercise lasts long enough, say 10 minutes, complete diffusion of lactic acid appears to take place between blood and tissues so that the lactic acid concentration of the blood at the end of exercise may be considered proportional to the
amount of lactic acid present in the whole body at that time. The relation between such a value of lactic acid concentration and oxygen debt, as calculated following A. V. Hill's procedure, shows that the lactic acid value remains at the resting level until the oxygen debt reaches the value of about 3.5 liters, after which a linear relationship exists between the two functions. This shows that until an oxygen debt of 3.5 liters is reached, corresponding in our experimental conditions to a work output involving two-thirds of the maximum metabolic rate, no extra lactic acid production occurs. For higher oxygen debts the increase of lactic acid production is 5 mols for every 3 mols of increase of the O₂ debt, which corresponds to a ratio between lactic acid burned and lactic acid removed of 1:5; this involves the assumption that in such conditions all the excess oxygen is used up in the removal of the lactic acid.

The analysis of the oxygen debt curve in recovery shows that this may be interpreted as the sum of three curves, two of which follow a logarithmic decrement, while the third is linear. Of the two logarithmic curves, one may be attributed to the lactic acid mechanism of paying the oxygen debt, as the lactic acid concentration in the blood decreases logarithmically: in our subject having a maximum O₂ debt of about 11.5 liters, a maximum of 8 liters may be attributed to this mechanism. The recovery with this mechanism is very slow, the speed constant being 0.02 to 0.03. The other logarithmic curve shows the existence of another mechanism of paying the O₂ debt, which cannot be attributed to the lactic acid: it is a much faster process, the speed constant being about 0.5. This fraction of the O₂ debt is practically completely paid in the first 3 to 4 minutes of recovery. The amount of O₂ debt which can be contracted in this way is, however, smaller than with the lactic acid mechanism. The linear function seems to be due chiefly to an increase of the metabolism of muscles after exercise and it has nothing to do then with a real oxygen debt. This hypothesis is confirmed also by the fact that it is not a logarithmic function as expected (an expectation confirmed by the other two mechanisms) and by the fact that after hard exercise the resting metabolism keeps higher for several hours after all the chemical and physicochemical variations detectable come back to normal.
The hypothesis is advanced that extra lactic acid formation in muscles does not occur in moderate exercise but is only a reserve mechanism brought into play during hard work in anaerobic conditions.

AN INSECT TEST FOR VITAMIN B FRACTIONS

By C. M. McCAY

(From the Laboratory of Animal Nutrition, Cornell University, Ithaca)

Insects lend themselves to nutrition studies because large numbers can be employed in experimental groups. Many variables can be studied simultaneously with economy of space and materials. Their great disadvantage is their ability to select food particles from diets prepared in the usual manner.

In our studies we have employed the common household pest, Blattela germanica, which is better known as a cockroach. We have tested a number of stock diets for rearing this insect. It can pass through its entire life cycle upon a diet of equal parts of dry skim milk and whole wheat. It can grow for a considerable period upon a product such as linseed oil meal but it cannot reproduce.

In our first series of experiments we employed purified diets such as those used for vitamin A assays with rats. We discovered that cockroaches could not grow upon a diet of cooked starch 22, cellulose 2, lard 10, sugar 10, mineral mixture 6, and casein 40. On the other hand, if this diet is supplemented with 5 parts each of cod liver oil and dried yeast, this insect species will grow almost as rapidly as it will when fed the stock diet of wheat and skim milk. At a temperature of 25° adult size is attained in about 60 days if a complete diet is fed.

A hundred experiments were run to determine some of the nutritional factors that limit the growth of cockroaches. Thus far growth, alone, has been studied without regard to the completion of the reproductive cycle. We have obtained no evidence that this species needs either vitamin A or D. It must have one or more of the vitamin B factors in yeast, however. This vitamin B fraction in yeast is soluble in 95 per cent ethyl alcohol.

Two series of experiments were run to compare zein, gelatin, and casein as sources of protein when fed at 20 per cent levels. An alcoholic extract of yeast furnished the vitamin B required.
Good growth was obtained with the casein, moderate growth with gelatin, and very poor growth with zein. These results indicate that this insect reacts to both deficiencies in vitamin B factors and in amino acids.

FURTHER STUDIES OF SYNTHETIC DIETS FOR HERBIVORA

BY C. M. McCAY, L. L. MADSEN, AND L. A. MAYNARD

(From the Laboratory of Animal Nutrition, Cornell University, Ithaca)

A previous paper by C. M. McCay, L. L. Madsen, and L. A. Maynard from this laboratory reported preliminary studies showing that a synthetic diet satisfactory for rats caused paralysis of the hind legs and death in rabbits, with a histological picture showing muscular degeneration. Goats placed on a synthetic diet at weaning were growing normally at the date of the previous report. Five of the six animals have now died after periods on the diet ranging from 140 to 190 days of nearly normal growth. In each case death was sudden with no symptoms of paralysis, but muscle lesions were found on histological examination. A degeneration of the heart muscle was found in all cases. The sixth goat is still thriving after 306 days on the diet.

In continuing the rabbit studies, with a diet consisting of regenerated cellulose 20, corn-starch 40, casein 15, sucrose 10, yeast 5, salt mixture 4, lard 4, and cod liver oil 2, with tomato juice fed separately, it has been found that the addition of alfalfa prolongs the growth and survival period. The entire substitution of alfalfa after loss of weight and paralysis have set in overcomes these troubles. The substitution of an A-D concentrate for the cod liver oil in the synthetic diet prolongs the survival period. Evidence that one of the faults of the synthetic diet is its physical nature is furnished by the frequent finding of impaction in the intestine. In preliminary experiments with a few guinea pigs, with the same synthetic diet, the same paralysis and muscular lesions found in the rabbit have developed.

These studies are being continued in order to develop a satisfactory synthetic diet for Herbivora and to reveal the cause of the

62 This finding was made by Dr. Peter Olafson of the New York State Veterinary College.
muscle dystrophy and other anatomical changes which are apparently of nutritional origin.

THE STABILITY OF CAROTENE IN ETHYL ESTERS OF FATTY ACIDS, LIVER, AND VEGETABLE OILS

By FRANCIS G. MCDONALD

(From the Research Laboratory, Mead Johnson and Company, Evansville, Indiana)

Solutions of carotene in ethyl butyrate, ethyl laurate, ethyl palmitate, cod liver oil, maize oil, peanut oil, and Wesson oil were stored in partially filled, tightly stoppered brown bottles at 37°, 24°, and 5°. Similar solutions sealed in ampules under a vacuum were stored at 37°. The esters were highly purified and the oils were good commercial grades.

From time to time the solutions were sampled and carotene determinations carried out with the aid of a spectrograph and sector photometer by observing the extinction limits of the 462 m\(\mu\) carotene absorption band.

At 37° in bottles practically all of the carotene disappeared within 2 weeks in the esters and peanut oil, and in 8 weeks more than 60 per cent in the other oils. At 24° the decomposition was slightly retarded. At 5° the loss of carotene in the esters and peanut oil amounted to 80 per cent in 4 weeks. In cod liver oil, Wesson oil, and maize oil only 8 per cent of the carotene was lost in 4 weeks. Storage of the solutions in sealed ampules under a vacuum resulted in somewhat better protection in the solvents of low protective power.

BENCE-JONES PROTEIN

I. CHEMICAL COMPOSITION

By GRACE MEDES

(From the Department of Medicine, University of Minnesota Medical School, Minneapolis)

Bence-Jones protein was isolated from the urines of five patients with multiple myeloma. The present paper deals with methods of isolation and purification of these proteins and a comparative study of their chemical compositions. The determinations in-
cluded ash, phosphorus content, water content, nitrogen distribution as determined by the Van Slyke method, and the tryptophane and tyrosine content. A preliminary study of their behavior in the presence of some salting-out agents was also made.

VARIATIONS IN THE PLASMA PHOSPHOLIPID OF AMYTALIZED DOGS FOLLOWING EPINEPHRINE OR INSULIN

BY LLOYD C. MILLER

(From the Department of Biochemistry and Pharmacology, The University of Rochester School of Medicine and Dentistry, Rochester, New York)

Data indicating marked increases in “blood fat” following epinephrine injections in amytalized dogs have been published by Himwich and Spiers. Long and Venning have recently shown that this alleged increase is an artifact arising from the titrimetric method used in determining the plasma fatty acids.

In this work, the experiments of the former authors have been repeated and extended. In addition to determining total fatty acids, both free and total cholesterol and phospholipid have been studied by methods developed in this laboratory. While no significant changes have been noted in the total fatty acids or cholesterol fractions following epinephrine or insulin administration in the amytalized dog, striking variations in the plasma phospholipid fraction have been observed. The effects of these hormones seem to be enhanced by if not dependent on the amytal anesthesia, since only inconsistent effects are seen in the unanesthetized dog. No effect is seen in the untreated amytalized dog.

Except for time relationships, these changes in plasma phospholipid closely resemble those produced concomitantly by epinephrine and insulin on the blood sugar. An average increase of 60 per cent over the initial level is seen in the plasma phospholipid 3 hours after the injection of mildly hyperglycemic doses of epinephrine. An average decrease of 70 per cent of the initial level is seen 3 hours after the injection of hypoglycemic doses of insulin. These relative changes in amount appear to be inde-

pendent of the height of the initial level of plasma phospholipid and of the degree of change in the blood sugar.

THE EFFECT OF MODERATE DOSES OF VIosterol AND OF PARA-
THYROID EXTRACT UPON BONE COMPOSITION

By Agnes Fay Morgan, Louise Kimmel, Rachel Thomas, and Zdenka Samisch

(From the Laboratory of Household Science, University of California, Berkeley)

Separate ash analyses were made of metaphyses and shafts of femora and tibiae of 137 rats fed a diet of normal Ca and P content and ratio, with varying amounts of viosterol, from 0 to 1500 D, and in some cases with and without moderate doses of parathyroid extract. The ash content of the metaphyses was slightly greater in the groups without vitamin D or with moderate amounts as compared with those of groups which received the larger doses, but less difference could be seen in the ash contents of the shafts of any of the groups examined. The parathyroid treatment likewise decreased the ash content of the metaphyses but did not affect the shafts.

Calcium and phosphorus balances were more favorable in the animals which received the vitamin, 10 D, than in those which did not. On injection of parathyroid extract these retentions were much decreased in both cases and somewhat more so in the group receiving both the extract and the vitamin. The increase in calcium content of the kidneys which was seen in the animals receiving moderate amounts of viosterol and of parathyroid extract as compared with that of the untreated groups was considerably greater in the parathyroid-treated rats both with and without the viosterol.

These metabolism and bone composition findings are taken to mean that therapeutic doses of viosterol favor rapid shaft formation at the expense of deposit of trabeculae in the metaphyses and that moderate doses of parathyroid extract may deplete the metaphyses of these trabeculae without affecting the shafts. The increased losses in balances and metaphysis ash seen in animals which received both substances appear to indicate that the effects of the vitamin and of the hormone are similar and cumulative.
Since the effect of experimental conditions upon bone ash may be more strikingly demonstrated by examination of the metaphysis alone than by the use of the whole femur, the routine use of the metaphysis is suggested for vitamin D assay and similar studies.

THE OXYGEN CAPACITY OF HEMOGLOBIN OF HUMAN BLOOD

BY DEMPSIE B. MORRISON

(From the Department of Chemistry, University of Tennessee College of Medicine, Memphis)

Two methods of determining the oxygen capacity of hemoglobin of human blood are employed.

The first method involves a determination of the oxygen to iron ratio in whole blood, and calculation from this ratio and the iron content of purified dry hemoglobin of the oxygen capacity of the latter. The oxygen capacities of fourteen samples of human blood have been measured by the carbon monoxide method of Van Slyke and Hiller, and the iron values by a potentiometric method with the use of either titanous chloride or titanous sulfate. The average oxygen capacity per 1 gm. of iron was 400 cc. ± 5 cc. (theoretical value, 401 cc.). Various methods of purifying hemoglobin were employed. The iron content of dried samples thus prepared varied from 0.305 to 0.338 per cent, depending upon the method of preparation and the conditions of drying. Samples dried in air at 40°, and at room temperature over phosphorus pentoxide, gave lower but more constant values than samples dried in air at 110°. At the latter temperature some samples were so charred that they were discarded.

The second method depends upon measuring the oxygen capacity of purified hemoglobin solutions, and direct estimation of the dried hemoglobin prepared from aliquots of these solutions. The first and, so far, the only determination by this method has given the value of 1.30 cc. of oxygen per 1 gm. of dry hemoglobin.

VI. THE LABILITY OF HYDROXYPROLINE IN THE MOLECULE OF PROTEINS

By Withrow Morse

(From the Röhm and Haas Company, Inc., Philadelphia)

In applying the color reaction for hydroxyproline which we have reported elsewhere,\textsuperscript{67} it became evident that hydroxyproline was readily loosened from the molecule. In the shearing action of the collagen prepared in a purified state,\textsuperscript{68} the protein was so affected that simple washing of the shredded collagen removed enough of the hydroxyproline to give a clearly positive test. Trypsin loosened hydroxyproline in a similar manner. Leather, however, even after its drastic treatment in various media, retains the capacity for giving a positive test for hydroxyproline; evidently the tanning process fixes the protein in such a manner that hydroxyproline is retained. We have studied the hydroxyproline content of ten commercial preparations of gelatin and these fall into two groups; namely, one giving the positive reaction, the other failing to give it. From our own experiments in preparing gelatin from collagen, we believe the reason for this grouping is that in one set hydroxyproline is retained in the medium which is not removed during manufacture and accompanies the finished product (Knox gelatin type) but is discarded in the other (Coignet type) which, therefore, fails to give a positive test.

CHANGES IN SERUM CALCIUM AND PHOSPHORUS AND IN THE TEETH DURING PREGNANCY

By James W. Mull and F. M. Kinney

(From the Laboratories of the Maternity Hospital and the School of Dentistry, Western Reserve University, Cleveland)

A study of changes in serum calcium and phosphorus during pregnancy, and of their relation to the teeth, has been made on patients in our prenatal clinic by successive examinations of the teeth and blood during the course of pregnancy. Between the 28th week preceding delivery and the 7th week post partum, 4760

\textsuperscript{67} Morse, W., \textit{J. Biol. Chem.}, \textbf{100}, 373 (1933).
\textsuperscript{68} Morse, W., \textit{J. Biol. Chem.}, \textbf{97}, p. xxx (1932).
calcium determinations were made on 898 women. These data show that calcium is influenced by season, demonstrated by the fact that values found during the months of January to May are lower than those of the remaining months; and by pregnancy, as shown in the summer curve by a decline from an average of about 10.4 mg. to 9.9 mg. at 8 weeks before delivery, with recovery to around 10.0 mg. by term. Following delivery there is a sharp rise to 10.4 mg. which is maintained, even with nursing, to dismissal 6 weeks post partum. The winter curve, which is roughly parallel, lies entirely below the lower limits of the normal range, while the summer curve lies in the lower third.

Phosphorus shows no seasonal variation. There is a slight fall which reaches a maximum about 10 weeks before delivery, with gradual recovery until delivery, followed by a marked rise, which is maintained throughout the period of observation.

Dental examinations were made on 358 patients. Oral findings, checked whenever possible by x-ray and confirmed in every instance, showed change during pregnancy in only 54 patients, 15 per cent. Of the 215 returning for post partum check up only 6 per cent showed change.

Forty-nine of the 54 cases showing dental decay had a total of 354 blood determinations, which showed them to fall within the normal range established by our study. We conclude, therefore, that serum calcium and phosphorus bear no direct relation to the pregnant woman's teeth.

A REPORT ON THE DEVELOPMENT, PREPARATION, AND DISTRIBUTION OF THE INTERNATIONAL VITAMIN STANDARDS

By E. M. Nelson

(From the Protein and Nutrition Division, Bureau of Chemistry and Soils, United States Department of Agriculture, Washington)

International standards for vitamins A, B, C, and D were adopted by the Health Committee of the League of Nations at a meeting in London on June 17 to 20, 1931. Standard preparations for three of these vitamins, A, B, and D, have been prepared and are being distributed to a central agency in each country by the Department of Biological Standards of the Medical Research Council of London. An allotment of these preparations for this country has
been received by the Bureau of Chemistry and Soils for distribution in this country.

THE EFFECT OF DIETS RICH IN CHOLESTEROL ON THE TISSUE LIPIDS OF YOUNG RATS

BY RUTH OKEY

(From the Laboratory of Household Science, University of California, Berkeley)

Young rats were placed at weaning upon adequate purified diets containing 15 per cent Crisco as the source of fat. Cholesterol to the extent of 1 per cent was incorporated into the diet of the experimental group, while the diet of the controls was nearly sterol-free. The rats were killed at the age of 3 months. Livers of the experimental animals were whitish, weighed approximately 1.5 times as much as those of the control groups, and contained only 50 per cent moisture as contrasted with 64 per cent for the controls. The free cholesterol content of the livers of the two groups was nearly the same and normal. Cholesterol as ester was, however, over 6.0 per cent in the livers of the cholesterol-fed rats and only approximately 0.15 per cent in the control group; "total lipid" and lecithin of the livers of the cholesterol-fed rats, 30.7 per cent and 2.4 per cent respectively; while the values for the control group were 12.0 per cent "total lipid" and 3.3 per cent lecithin.

In a group of the rats from which the vitamin B complex (as yeast extract) was withdrawn 10 days before killing, the cholesterol ester of the livers was approximately one-third lower, while the food intake had remained very nearly constant.

Other tissues are still under investigation. The work is being repeated at different levels of fat intake.

THE PREPARATION OF A CRYSTALLINE GLOBULIN FROM THE ALBUMIN FRACTION OF COW'S MILK

BY A. H. PALMER

(From the Department of Chemistry, New York University and Bellevue Hospital Medical College, New York)

By prolonged dialysis under carefully controlled conditions of pH, a crystalline globulin may be separated from the albumin frac-
In the production of cow's milk. It has been obtained in yields approximating 50 per cent of the total "albumin," is readily recrystallizable, and appears to be a homogeneous and reproducible product. It is insoluble in water within a rather narrow range of pH, and in high concentrations of sodium and ammonium sulfates. It is soluble in very dilute solutions of acids, bases, and salts. Its temperature of coagulation is about 80° at pH 5.5. The solubilities of the product in dilute and in concentrated solutions of salt are a simple function of the ionic strength of the solvent.

REFLECTION IN THE RAT

BY HELEN T. PARSONS, EUNICE KELLY, AND DOROTHY L. HUSSEMANN

(From the Department of Home Economics, University of Wisconsin, Madison)

Bulky white feces containing numerous starch grains, similar to feces reported by others as typical of "refection," occurred spontaneously in individual rats on a vitamin G-low ration containing raw potato starch, sucrose, casein, salt mixture, cod liver oil, and 6 per cent of rice polish. This refection was transmitted to young rats on a similar ration containing 2 per cent of rice polish. The refection was accompanied by a marked protection from the inadequacy of the diet, three refected rats being still alive with a body weight of over 230 gm. at the end of 14 months, while the non-refected individuals from the same litter died in from 29 to 55 days on the ration. Reflection was lost and the rats died if cooked potato starch was substituted for the raw.

There has been a spontaneous appearance of white feces in rats whose ration contained raw corn-starch as the sole carbohydrate. By feeding these white feces to a second group of rats a change in color and size of their feces could be induced for varying lengths of time, although the animals seemed to be protected little, if at all, by this addition to the ration. Starch grains were not detected in these feces.
THE CURE OF DERMATITIS DUE TO EGG WHITE BY VARIOUS FOODSTUFFS

BY HELEN T. PARSONS, JANE G. LEASE, AND EUNICE KELLY
(From the Department of Home Economics, University of Wisconsin, Madison)

Dried yeast, dried egg yolk, wheat embryo, and dried milk are only moderately effective in curing egg white dermatitis, since a concentration from 1 to 3 times that of the toxic egg white in the diet is necessary to be curative.

Cooked beef liver, pork liver, and beef kidney are effective if present in a concentration one-fourth that of the egg white. Cooked pig kidney is nearly twice as potent as beef liver.

There is relatively little or no potency in spleen, heart, ovaries, adrenals, blood, hemoglobin, or Eli Lilly and Company liver extract No. 343. The potency of liver does not depend on its nucleoprotein fraction.

The activity of raw liver or kidney is increased by cooking. Potency is not decreased significantly by autolysis, by prolonged bacterial action, or by standing at room temperature. The activity is decreased by boiling for 1 hour with hydrochloric acid at a concentration of 5 or more per cent; it is destroyed by heating cooked liver for 6 days at 100°.

Small single doses of kidney lead to very effective cures when given, after a 1 day's fast, to rats with pronounced dermatitis.

THE RÔLE OF OXIDATION AND REDUCTION PROCESSES IN THE ACTIVITY OF UREASE

BY WILLIAM A. PERLZWEIG
(From the Department of Biochemistry, Duke University School of Medicine, Durham)

Urease, in crude extracts and in solutions of the crystalline preparations, is susceptible to loss of activity on exposure to atmospheric oxygen or to other oxidizing reagents, such as H₂O₂, catechol, and other polyhydric phenols. Presumably heavy metals catalyze this oxidation. When such an inactivation is only partial, with loss of not more than one-half of the activity, it may be easily reversed by reducing reagents, such as cyanides and R—SH compounds. Urease, when more completely inactivated by ox-
dation, cannot be reactivated in this manner. Apparently the inactivation of urease proceeds in at least two distinct stages.

**NITROGEN CONSTITUENTS OF MOLD MYCELIUM**

**By W. H. Peterson, H. J. Gorcica, and E. B. Fred**

(From the Departments of Agricultural Chemistry and Agricultural Bacteriology, University of Wisconsin, Madison)

The fresh mycelium (containing 5.1 per cent nitrogen, dry basis) of *Aspergillus fischeri* was fractionated into four parts according to solubility: water-soluble 31, lipid 1, alkali-soluble 53, and insoluble 12 per cent of the total nitrogen.

Most of the nitrogen in the water-soluble fraction was present as α-amino nitrogen. This fraction gave a negative biuret test, and positive xanthoproteic, Millon, and Hopkins-Cole tests. No precipitate resulted from the addition of mineral acids or trichloroacetic acid, but a heavy precipitate was obtained with Ba(OH)₂ or with alcoholic KOH.

The alkaline extract gave positive biuret, xanthoproteic, Millon, and Hopkins-Cole tests. About one-fourth of the alkali-soluble nitrogen was precipitated by acid. This precipitate contained 10 to 12 per cent nitrogen, 1 to 2 per cent ash, and reduced Fehling's solution after acid hydrolysis. The largest part of the alkali-soluble nitrogen was peptone-like in character. It was not precipitated by acids or alcohol, but was precipitated by tungstic acid, or by saturation of its solution with zinc sulfate.

The rest nitrogen, insoluble in water and alkalies, was part of a complex polysaccharide. After acid hydrolysis, 62 per cent of this nitrogen was isolated as glucosamine nitrogen.

**THE CORTICAL HORMONE REQUIREMENT OF THE ADRENALECTOMIZED DOG**

**By J. J. Pfiiffer, Harry M. Vars, P. A. Bott, and W. W. Swingle**

(From the Biological Laboratory, Princeton University, Princeton)

A series of seven adrenalectomized dogs (8 to 14 kilos) was allowed to come into insufficiency by gradual reduction of dosage at 7 to 10 day intervals. These animals had been adrenalectomized
2 to 10 months previous to the start of the experiment and had been in insufficiency one to six times. A single preparation of hormone was used. The successive levels of hormone therapy employed were as follows: 0.1, 0.05, 0.025, 0.0125, 0.01, 0.0084, 0.0076, 0.006, 0.0055, and 0.005 cc. per kilo per day. The clinically failing dose ranged from 0.005 to 0.0084 cc. (0.2 to 0.34 gm. of whole beef adrenal gland) per kilo per day. The clinical maintenance dose ranged from 0.0055 to 0.01 cc. The dose on which a rise of 100 per cent in the blood urea nitrogen was observed ranged from 0.006 to 0.01 cc.

Five animals of this series were used to repeat the above assay under different dietary conditions. Two animals received the same diet as in the above experiment and gave comparable results. Two animals received a diet less palatable and exhibited insufficiency at a slightly higher level. One animal receiving beef heart plus supplements failed on a dose twice as large as previously. The anorexia, and its accompanying physiological manifestations, can explain the earlier failure of animals subsisting on poorly relished diets.

One animal died of adrenal insufficiency during the experiment while on a clinically failing dose. One animal has since died with typical symptoms on withdrawal of extract. The remaining five animals are being used in further work and have since been in insufficiency from two to five times.

THE MANIFESTATION OF SCURVY-LIKE SYMPTOMS INDUCED BY THE INGESTION OF SODIUM FLUORIDE*

By PAUL H. PHILLIPS

(From the Department of Agricultural Chemistry, University of Wisconsin, Madison)

The influence of sodium fluoride upon guinea pigs fed scorbutic diets fortified with graded levels of orange juice has been studied. It has been found that several times the normal antiscorbutic dose of orange juice will not prevent the appearance of symptoms similar to, if not identical with, those of scurvy when 25 to 30 mg. of fluorine per kilo of live weight are fed. It is tentatively suggested

* Presented before the American Society for Experimental Pathology.
that chronic fluorine poisoning may be an interference with the action of vitamin C in the organism.

THE USE OF A MODIFIED KROGH-REHBERG APPARATUS FOR THE DETERMINATION OF CARBON DIOXIDE IN BONE POWDER

BY MARSCHELLE H. POWER AND MILDRED ADAMS

(From the Section of Clinical Metabolism, The Mayo Clinic and The Mayo Foundation, Rochester, Minnesota)

The vacuum extraction technique of Krogh and Rehberg for liquids and tissues has been applied to the determination of carbon dioxide in dry substances, such as bone powder. The results are in satisfactory agreement with those obtained by the use of the Van Slyke-Neill manometric apparatus, and the technique as a whole presents some advantages over the latter procedure as regards ease of introduction of sample and subsequent manipulation, simplicity of apparatus, and flexibility.

THE LIPID CONTENT OF CERTAIN MOLDS

BY L. M. PRUSS AND F. M. STRONG

(From the Departments of Agricultural Chemistry and Agricultural Bacteriology, University of Wisconsin, Madison)

The lipid contents of twenty-four molds grown on both synthetic (glucose-inorganic salts) and organic (glucose-malt sprouts) media have been determined. The percentage of lipid varied with the species, the amount of aeration, and the reaction and composition of the medium. On the synthetic medium the lipid percentage of the mycelium ranged from 1 to 20, average 6.0, and on the organic medium from 1.5 to 25, average 8.8. In the majority of cases, on both types of media, the lipid percentage was markedly increased when aeration was restricted (lead foil caps over the cotton plugs of the flasks). Other factors which may influence the lipid content, e.g. temperature of incubation, glucose concentration of the medium, etc., are under investigation.

The character of the lipids of Aspergillus sydowi was studied by separating 1.23 kilos of material, obtained by extraction of 16 kilos of dried mycelium with acetone and alcohol-ether, into va-
rious fractions. Approximately 10 per cent of the lipid was a phosphorus- and nitrogen-containing substance, apparently different from well known phospholipids. The remaining simple lipids contained about 7 per cent of unsaponifiable material and 80 per cent fatty acids. About 25 per cent of the acids was present in the free state and 75 per cent in the combined form, while 28 per cent was found to be saturated and 66 per cent unsaturated (iodine numbers 115 to 120).

THE EFFECT OF DESICCATED THYROID FEEDING AND PARATHYROID HORMONE INJECTION UPON THE EXCRETION OF CALCIUM IN THE NORMAL AND HYPOPHYSECTOMIZED RAT

By L. I. PUGSLEY

(From the Department of Biochemistry, McGill University, Montreal, Canada)

The feeding of desiccated thyroid to normal rats caused a marked increased excretion of calcium in the feces and only a slight increase in the urine. When parathyroid hormone was administered following thyroid medication, a summation effect occurred in the calcium excretion. The hypophysectomized rat responded like the normal rats to desiccated thyroid and to parathyroid hormone.

THE RÔLE OF PARATHYROID HORMONE IN THE RAT

By L. I. PUGSLEY and HANS SELYE

(From the Department of Biochemistry, McGill University, Montreal, Canada)

The continued administration of parathyroid hormone to rats was found first to lead to the formation of numerous osteoclasts, hypercalcemia, and increased calcium excretion, but later this process was reversed and numerous osteoblasts appeared and a decreased calcium excretion.
STUDIES ON THE ACID-BASE CONDITION OF BLOOD

III. THE pK' OF HUMAN AND DOG SERA

BY HOWARD W. ROBINSON, J. WAIDE PRICE, AND GLENN E. CULLEN

(From the Children's Hospital Research Foundation and the Department of Pediatrics, College of Medicine, University of Cincinnati, Cincinnati)

Method—In former determinations69 of the constant pK' of the Henderson-Hassellbalch equation

\[ \text{pH} = \text{pK'}_{1} + \log \frac{[\text{CO}_2] - [\text{H}_2\text{CO}_3]}{[\text{H}_2\text{CO}_3]} \]

the Clark-Cullen hydrogen electrode vessel was employed. The serum was equilibrated at a known CO₂ tension and a hydrogen-CO₂ mixture of the same CO₂ content was used in the electrode vessel. The pH and CO₂ content determinations were therefore made on samples of serum separately equilibrated, and regardless of careful technique the accuracy of the comparison depended on the matching of these two CO₂ tensions. The present study presents a method by which the pH and CO₂ content are determined consecutively on the same equilibrated serum, the bubbling hydrogen electrode vessel of Simms,60 with a constant mixture of CO₂ (5.56 per cent) and H₂, being used. Adequate precautions to secure temperature constancy were taken. To prevent foaming of the serum a drop of octyl alcohol is added. This has no effect on the pH of phosphate standards but does produce a turbidity in the serum that apparently does not interfere with any determination. At 38° equilibration is obtained in from 15 to 20 minutes. After obtaining constant E.M.F. readings the platinum electrode is removed and a sample of equilibrated serum is drawn directly into a Van Slyke-Ostwald pipette for the [CO₂] determination in the Van Slyke manometric apparatus. From 2 to 4 cc. are required for the complete analysis. In the calculation of pK'₁, the value for

\[ \alpha \text{CO}_2 \text{ at } 38^\circ \text{C of } 0.510 \text{ of Van Slyke et al. was used.} \] This technique gives a rapid, convenient, and accurate method for the determination of pK'.

**Results**—Over 70 determinations of pK' have been made on dog sera and twenty on human sera. In confirmation of the results of Cullen, Keeler, and Robinson the pK' values for human sera average practically the same as those for dog sera. The range is from 6.072 to 6.115 with a mean for 95 determinations of 6.092 and standard deviation of ±0.005. This distribution about the mean is much more satisfactory than in any previous results. This value is slightly lower than the average pK' value of 6.10 recommended by Hastings, Sendroy, and Van Slyke.

Ten determinations were made on each of four apparently normal dogs over a period of 8 months. The variation of pK' of an individual dog is as great as is the variation of the group. Following various experimental procedures including parathormone, histamine with withdrawal of gastric secretion by stomach tube, diphtheria antitoxin, ammonium chloride, mercuric chloride, excessive doses of irradiated ergosterol, and ether and sodium barbitol anesthesia, the pK' of the dog sera varied no more than did that of the normal dogs.

The human series includes sera from bloods of normal humans, from patients without any known disturbance of acid-base condition, and from patients with nephrosis, nephritis, and renal rickets.

**STUDIES ON THE ACID-BASE CONDITION OF BLOOD**

**IV. THE C CORRECTIONS OF THE COLORIMETRIC pH METHOD FOR PLASMA AND SERUM**

BY HOWARD W. ROBINSON, J. WAIDE PRICE, AND GLENN E. CULLEN

(From the Children's Hospital Research Foundation and the Department of Pediatrics, College of Medicine, University of Cincinnati, Cincinnati)

The method described in the preceding communication in which the bubbling electrode was used in determining the pK' of sera, is also applicable to the determination of the C correction, that is, the difference between the electrometric and colorimetric determinations. \( C = [pH]_{20^\circ} - pH_{43^\circ} \) where the brackets indicate colorimetric reading and the subscript e the electrometric.
It is particularly advantageous not only because the colorimetric and the electrometric determinations are made from the same sample but because the pK' value which was obtained coincidentally gives an index of any abnormality in the electrolyte condition.

C corrections were determined on the sera for which pK' values were given in the preceding paper. The range of distribution for dog sera is from 0.24 to 0.40 with a mean of 0.31. In sera from human blood which is apparently normal the range is from 0.28 to 0.31. In human serum from patients with kidney diseases in which the albumin is below 4 per cent the C correction range is from 0.21 to 0.25. In a few cases where C corrections from serum and plasma from oxalated blood have been obtained at the same time the plasma C correction is invariably lower than that of the serum. This observation taken with the original report of Cullen that the C correction of horse plasma is less than that of horse serum suggests that many of the apparent discrepancies of C correction in normal human blood have been due to the fact that within the last few years serum has been studied rather than plasma with the assumption, which we now believe to be incorrect, that the corrections are the same.

THE DISAPPEARANCE OF HEXOSEMONOPHOSPHATE FROM FROG MUSCLE

BY ETHEL RONZONI AND MARGARET KERLY

(From the Laboratory of Biological Chemistry, Washington University School of Medicine, St. Louis)

In common with others (Cori and Cori, Buell, Strauss, and Andrus) we find that hexosemonophosphate increases under anaerobic conditions. At pH 6.0 in a CO₂-bicarbonate buffer hexosemonophosphate (Emden ester) of intact resting muscle is increased from 30 to 70 to 80 mg. per cent as hexose in 2½ hours at 25° with practically no production of lactic acid. It was of interest to examine the behavior of this excess hexosephosphate formed

under conditions in which no lactic acid is produced, in contrast to that accompanied by lactic acid formation as in muscle contraction, investigated by Cori and Cori. We find that changing the pH to 9 by substituting nitrogen for CO₂ restores the ability to produce lactic acid but causes only insignificant changes in hexosemonophosphate. If CO₂ is replaced by O₂ containing 5 per cent CO₂, bringing the pH to 7, a small amount of lactic acid, already low, disappears and hexosemonophosphate disappears at a dearly constant rate for a period of 3 hours, averaging 16 mg. per cent in 1 hour, 38 mg. per cent in 2 hours, and 55 mg. per cent in 3 hours. There is no evidence from these experiments that this hexosephosphate is an intermediate in lactic acid formation.

CERTAIN BLOOD CHANGES ASSOCIATED WITH PHYSICAL EXHAUSTION

BY FREDERIC W. SCHLUTZ, A. BAIRD HASTINGS, AND MINERVA MORSE

(From the Lasker Foundation for Medical Research and the Departments of Pediatrics and Medicine of the University of Chicago, Chicago)

Observations of the changes in the blood sugar, lactate, and acid-base balance have been made on dogs brought to physical exhaustion by swimming at 38°.

The following results have been obtained. (1) After 15 minutes of swimming, the acid-base balance is displaced in the direction of fixed acid excess. This is due in large measure to the entrance of lactic acid into the blood steam. The serum sugar is usually also elevated. (2) During the subsequent swimming period, varying from 1.5 to 4.5 hours, prior to the onset of exhaustion, there is usually a decrease in the serum lactate and a return of the acid-base balance toward normal. The serum sugar is usually normal in this period. (3) With the onset of exhaustion, there is a rise in serum lactate and displacement of the acid-base balance in the direction of fixed acid excess. (4) During the recovery period, the excess lactate rapidly decreases and is practically normal after 30 minutes rest. The acid-base balance returns toward normal. (5) Increasing the intensity of the work done causes an increase in serum lactate. (6) The higher the lactate concentration, the sooner the

onset of exhaustion. (7) Serum total base, chloride, and phosphates showed no significant change in this series of experiments.

FURTHER STUDIES ON THE RELATION OF THYROID ACTIVITY TO THE POWER OF CERTAIN BILE SALTS TO PRODUCE GASTRIC ULCERS

By L. H. SCHMIDT

(Christ Hospital Research Fellow in Biochemistry, University of Cincinnati, Cincinnati)

Ingestion of thyroxine by a male guinea pig increases the toxicity of a mixture of the sodium salts of taurocholic and glycocholic acids. This ingestion also increases the capacity of injections of this bile salt mixture to produce lesions in the gastric mucosa (Tashiro and Schmidt64). The change in toxicity of the bile salts is roughly proportional to the amount of thyroxine ingested in the case of either male or female guinea pigs. However, the female animal is affected to a greater extent than the male by treatment with a certain amount of thyroxine. Although the minimum lethal and ulcer-producing dose of the mixture of bile salts is 0.0175 gm. per 100 gm. of weight for a normal male guinea pig and 0.0275 gm. per 100 gm. of weight for a normal female guinea pig, ingestion of 1.0 mg. of thyroxine reduces the amount of bile salt required for death and ulcer in either sex to 0.008 gm. per 100 gm. of weight.

Since Tsuruta and Ishii have demonstrated that lecithin, cephalin, and cholesteryl oleate inhibit bile salt toxicity and since ingestion of thyroxine produces decreases in blood phospholipid content in the rabbit, indications are that the above changes following ingestion of thyroxine are due to the stimulation of lipid metabolism. These indications are supported by the observation that the amount of lecithin required to inhibit death and gastric ulcer formation, following injection of a certain quantity of bile salt, is increased almost 3-fold by previous feeding of 1.2 mg. of thyroxine.

THE PRESENCE OF A NEW DIETARY PRINCIPLE IN LIVER

By WALTER H. SEEGERS AND H. GREGG SMITH

(From the Laboratory of Biochemistry, State University of Iowa, Iowa City)

Alcohol-extracted beef liver was fed to albino rats as the only source of protein in a ration adequate in the recognized factors necessary for normal nutrition, the vitamins being supplied by yeast, cod liver oil, and by hydrogenated cottonseed oil (Crisco), the latter furnishing the fat of the basal diet. The ration was found to be inadequate for optimum growth, reproduction, and lactation. Supplements of 300 mg. of a tested yeast vitamin concentrate (Yeast Vitamine-Harris) or a water-alcohol-soluble, ether-insoluble extract of the liver led to no significant improvement. Supplementing the basal diet with 0.5 gm. of raw liver or replacing the extracted liver of the basal diet with whole dried liver increased the growth rate and improved lactation. The total food consumed per gm. of increase in body weight was less when whole liver was fed, and the gains made per gm. of protein further indicate that the better growth is not the result of increasing the protein content of the diet. The animals fed whole liver had larger litters and weaned 30 per cent of their young, whereas none was weaned by the controls on the basal diet.

FAT METABOLISM AND THE LIVER LIPIDS

By ROBERT GORDON SINCLAIR

(From the Department of Biochemistry and Pharmacology, The University of Rochester School of Medicine and Dentistry, Rochester, New York)

In an effort to bring further evidence to bear on the widely held view that the fatty acids of depot or ingested fat, as an essential step towards combustion, are desaturated and built up into phospholipid in the liver, whence they are carried by the blood to the muscles and other organs, experiments have been carried out to determine the rate of turnover of the liver phospholipids.

If rats are raised on a standard ration which is low in fat or contains hardened coconut oil, the phospholipid fatty acids in the liver and other organs, and the depot fat, have characteristically low levels of unsaturation. If, on the other hand, the rats receive daily a small amount of cod liver oil in addition to the basic ration,
the phospholipid fatty acids have a comparatively high degree of unsaturation, whereas the fat deposited in the stores is unaffected.

It seemed reasonable to believe that a determination of the rate of fall in the iodine number of the phospholipids in the livers of these latter rats during fasting and following transfer to a diet containing hydrogenated coconut oil, should provide a measure of the rate of turnover of liver phospholipid. If the depot fat during fasting and the ingested hardened coconut oil are of necessity desaturated and built up into phospholipid in the liver, then the turnover of liver phospholipid should be very rapid and there should be a rapid fall in the iodine number.

The results obtained indicate that such is not the case. Even after many days of fasting or on a diet rich in hydrogenated coconut oil there is a relatively small and inconstant drop in the iodine number of the liver phospholipid fatty acids.

During fasting rather striking changes in the lipid content of the liver were observed. Together with the decrease in the actual weight of liver there was a decrease in the absolute amount of phospholipid, unsaponifiable material, and fat. However the percentage amount of both phospholipid and unsaponifiable material always increased, while that of the fat was rather irregular.

THE KINETICS OF CARBON DIOXIDE REACTIONS IN BUFFER SYSTEMS AND BLOOD

BY WILLIAM C. STADIE AND HELEN O'BRIEN

(From the John Herr Musser Department of Research Medicine, University of Pennsylvania, Philadelphia)

The velocity of the reactions of all forms of CO₂ with the constituents of a buffer system is dependent upon the slow reactions

\[
\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3, \quad (1)
\]

\[
\text{CO}_2 + \text{OH}^- \rightleftharpoons \text{H}_2\text{CO}_3, \quad (2)
\]

Toward the right, these equations express the hydration of CO₂; toward the left, the dehydration of carbonic acid. The velocity of the reaction (hydration or dehydration) is given by the equation

\[
-k_1a_{\text{CO}_2}^*a_{\text{H}_2\text{O}} - k_2a_{\text{H}_2\text{CO}_3}^*a_{\text{OH}^-} - k_3a_{\text{CO}_2}^*a_{\text{OH}^-} - k_4a_{\text{H}_2\text{CO}_3}^* \quad (3)
\]
Two general cases arise.

1. The reactions occur in infinite buffer; i.e., the pH is constant. Equation 3 may then be integrated and gives two equations, Equation 1 for hydration and Equation 2 for dehydration, which permit the calculation of the time necessary to reach any degree of equilibrium; e.g., 90 per cent from the experimentally determined values of the constants $k_1$, $k_2$, $k_3$, and $k_4$.

2. The reactions occur in dilute buffer; i.e., the pH is variable. Three cases arise.

1. $pH < 8.5$—The reaction given by Equation 2 becomes negligible.

   (a) Hydration of CO$_2$—The velocity is given by the equation
   \[
   - \frac{da_{\text{CO}_2}}{dt} - \frac{da_{\text{HCO}_3^-}}{dt} = k_1a_{\text{CO}_2}a_{\text{H}_2\text{O}} - k_2a_{\text{H}_2\text{CO}_3} - k_3a_{\text{CO}_2}a_{\text{H}_2\text{O}} \tag{4}
   \]
   This equation may then be integrated and the velocity constant $k_1$ determined experimentally from the changing values of $a_{\text{HCO}_3^-}$ against time.

   (b) Dehydration of CO$_2$—The velocity is given by the equation
   \[
   - \frac{da_{\text{H}_2\text{CO}_3}}{dt} = k_2a_{\text{H}_2\text{CO}_3} - k_1a_{\text{CO}_2}a_{\text{H}_2\text{O}} \tag{5}
   \]
   This equation may then be integrated and the value of $k_2$ determined experimentally from the changing values of $a_{\text{H}_2\text{CO}_3}$ against time.

2. $pH > 12$—The reaction given by Equation 2 then becomes negligible.

   (a) Hydration of CO$_2$—The velocity is given by the equation
   \[
   - \frac{da_{\text{CO}_2}}{dt} = k_2a_{\text{CO}_2}a_{\text{OH}^-} - k_4a_{\text{HCO}_3^-} \tag{6}
   \]
   which may be integrated and the value of the constant $k_2$ determined experimentally.

   (b) Dehydration of CO$_2$—At pH > 12 this is insignificant. For this reason the term $k_4a_{\text{HCO}_3^-}$ may be neglected in Equation 6.

3. $pH > 7.5 < 12$—The complete Equation 3 must be used. An integral exists but is too cumbersome for practical use.
Application to Blood—The values of the constants \( k_1 \) and \( k_2 \) extrapolated from 0° to 38° permit an approximate calculation of the time necessary for the blood to attain 90 per cent of equilibrium either in the uptake (hydration) or release (dehydration) of \( \text{CO}_2 \) in the tissues and lungs respectively. The calculated time is greatly in excess of the physiological demands and indicates some catalytic mechanism for these reactions in the blood. An active principle isolated from red blood cells was found to accelerate greatly the velocity of these reactions. The nature of this principle and the mechanism of its effects upon the velocity constants outlined above were studied and analyzed in detail.

STUDIES ON THE PHYSIOLOGY OF PYRIMIDINES. THE METABOLISM OF ISOBARBITURIC ACID IN MAN

BY JAKOB A. STEKOL AND LEOPOLD R. CERECEDO

(From the Department of Chemistry, Fordham University, New York)

The metabolism of isobarbituric acid in humans has been studied. We find that in man isobarbituric acid is partly metabolized to urea, partly excreted in conjugation with sulfuric acid.

In an attempt to determine in what manner various metabolic conditions would affect the fate of this compound in the human body, our subject was given only one meal every 24 hours. The 24 hour period was divided into three parts of 8 hours each; a digestive period (Period 1), beginning immediately after the meal, a postprandial period (Period 2), and a fasting period (Period 3). The urine passed during each of these three periods was separately analyzed for total nitrogen, creatinine, phosphorus, urea, and sulfur. We find that isobarbituric acid seems to be differently metabolized depending on the period in which it is ingested. The greatest variations are found when the substance is given at the beginning of Period 3.

We have obtained a strongly positive naphthoresorcinol test in the urine, indicating that isobarbituric acid causes an increased elimination of glucuronates.
METHODS FOR ESTIMATING THIOCYANATES IN URINE

By M. X. SULLIVAN

(From the Chemo-Medical Research Institute, Georgetown University, Washington)

The values obtained by the Rupp-Schied-Thiel iodometric method and the Munk gravimetric method applied to urine are too high, since other substances in urine react like thiocyanate in that they are precipitated by silver nitrate, react with iodine, or contain sulfur. Based on the fact that barium thiocyanate is soluble in alcohol and the interfering substances are not, an improved procedure was developed as follows: An aliquot of urine, generally 50 cc., is treated with a saturated aqueous solution of barium hydroxide as long as a precipitate forms. The mixture is filtered, evaporated, taken up in absolute alcohol, and the procedure of evaporating and dissolving in alcohol repeated. Finally the dry residue is dissolved in 25 cc. of water and the Rupp-Schied-Thiel procedure applied. The values found are much lower than when applied to urine unmodified.

THE ANALYSIS OF CHLORIDE IN TISSUES

By F. WILLIAM SUNDERMAN

(From the John Herr Musser Department of Research Medicine, University of Pennsylvania, and the Pennsylvania Hospital, Philadelphia)

Complete recovery of chloride in tissues was not obtained by the use of the direct open Carius method. A procedure for analysis, employing preliminary alkaline digestion followed by the open Carius method, has been developed which gives results agreeing within ±2 per cent with those obtained by established methods for analysis of chloride in organic materials.

THE NON-HYDROLYTIC OXIDATION OF CYSTINE

By GERRIT TOENNIES AND THEODORE F. LAVINE

(From the Research Institute of the Lankenau Hospital, Philadelphia)

The possibility of preparing compounds that derive from cystine by oxidation of the sulfur group and that are intermediate between cystine and cysteic acid was studied. The attempt was made to
dissolve and oxidize cystine in a non-aqueous medium, in order to
prevent hydrolysis of the \(-\text{S-S}\) linkage, which in aqueous oxida-
tion leads to the formation of cysteic acid. Cystine was found to
be soluble in alcoholic HCl solutions, but to undergo spontaneous
esterification therein. In anhydrous solutions of HClO$_4$ in acetic
acid a violent reaction takes place, in which a part of the cystine
is converted into cysteic acid. In an anhydrous solution of HClO$_4$
in acetonitrile, however, cystine is soluble in amounts fully equiva-

tent to the HClO$_4$, and such solutions are stable for several weeks at
room temperature. When oxidized in this solution by benzoyl
hydrogen peroxide, cystine takes up a maximum of 4 oxygen atoms,
and a precipitate has been isolated, the composition of which
approaches the formula \((\text{HOOC-CH(N$_2$)-CH$_2$-SO$_2$-})_2 + 1\text{HClO}_4\). The product is hygroscopic and in aqueous solution
undergoes hydrolysis as indicated by progressive acid formation.
The aqueous solution reduces iodine, but it is also capable of oxi-
dizing iodide if this is present in excess. While no \(-\text{SH}\) test is
produced by the aqueous solution with cyanide and nitroprusside,
the test appears after preliminary reduction by iodide.

A STUDY OF ANISOTROPIC SUBSTANCES IN THE URINE

BY MARY E. TURNER

(From the Chemical Laboratory, Babies Hospital, New York)

An investigation has been made of the chemistry and morphol-
ogy of the various anisotropic substances, particularly those struc-
tures showing a typical Maltese cross under the polarizing micro-
scope. Anisotropic lipid granules were prepared from cholesterol
esters in vitro and their variations studied. When sodium sali-
cylate or aspirin was administered to an individual, the urine con-
tained anisotropic granules having the same morphological forma-
tions as those of cholesterol ester. These granules were isolated
and identified as sodium salicylate. Cholesterol esters in the
urine may be overlooked owing to the various physical forms as-
sumed by the solid or liquid crystals, and non-lipid granules may
be interpreted as cholesterol esters. A microscopic test for choles-
sterol which aids in the study of lipid bodies in urine sediments has
been devised. The value of a chemical study combined with the
use of the polarizing microscope in the investigation of doubly
refracting lipids deserves emphasis.
MANOMETRIC MICRODETERMINATION OF CARBON IN ORGANIC SUBSTANCES

BY DONALD D. VAN SLYKE, IRVINE H. PAGE, AND ESBEN KIRK
(From the Hospital of The Rockefeller Institute for Medical Research, New York)

The method proposed is in principle the same used by Backlin. It has been changed in reagents, apparatus, and technique to increase speed and accuracy.

The sample is placed in a 15 cc. test-tube connected through a ground glass joint to the chamber of the Van Slyke-Neill manometric apparatus, and is burned by heating for 3 to 5 minutes with 1 cc. of a chromic acid mixture (12 to 15 gm. of chromic acid, 125 cc. of concentrated sulfuric acid, 125 cc. of concentrated orthophosphoric acid). By passing the gas from the combustion tube back and forth several times between the tube and the manometric chamber, all the CO$_2$ is absorbed in 2 cc. of 0.5 N sodium hydroxide in the chamber. The alkali solution is then acidified and the CO$_2$ extracted and measured manometrically as in blood CO$_2$ determinations. With the usual Van Slyke-Neill apparatus, and samples with 0.2 to 0.5 mg. of carbon, results are ordinarily accurate to within 1 part in 200. By using a manometric chamber calibrated to measure the CO$_2$ at 5 cc. instead of 2 cc. volume, with correspondingly larger samples, and by eliminating slight fluctuations in the blank due to CO$_2$ in the atmospheric air in the combustion tube, the usual error can be brought down to 1 part in 500. Good results are given by sugars, fatty acids, and amino acids. The simpler technique requires 11 to 12 minutes for a complete analysis; it has been found convenient for microdeterminations of lipids. The more precise modification requires 15 to 20 minutes.

OXIDATION PRODUCT OF PROTEINS, METHYLSULFONIC ACID

BY HARRY M. VARS
(From the Biological Laboratory, Princeton University, Princeton)

In 1914 Mörner reported the presence of methylsulfonic acid among the oxidation products of proteins after treatment with

nitric acid. Inasmuch as cystine is not converted to methylsulfonic acid by oxidation with nitric acid, he stated that his observation was strong evidence for the presence of an unknown sulfur-containing amino acid in proteins. No mention of Mörner's work has been made in papers concerned with the discovery and characterization of methionine.

Methylsulfonic acid in a yield approximating 70 per cent of the expected amount (on the basis of the non-cystine sulfur) has been obtained by the nitric acid oxidation of commercial casein. \((\text{CH}_3\text{SO}_3)_2\text{Ba}\), calculated, Ba 41.96, S 19.58; found, Ba 41.61, S 19.66.

A study of the oxidation of methionine is being made. An improved method of isolation of methylsulfonic acid is being developed, and purified proteins will be studied for their yield of this acid.

**SOME CHEMICAL PROPERTIES OF HIGHLY PURIFIED PREPARATIONS OF PITRESSIN AND PITOCIN**

By Vincent Du Vigneaud, Robert Ridgely Sealock, and R. H. Sifferd

(From the Department of Biochemistry, George Washington University, School of Medicine, Washington)

And Oliver Kamm and Irvine W. Grote

(From the Research Laboratories of Parke, Davis and Company, Detroit)

A series of preparations of pitressin and pitocin of varying degrees of potency have been studied to detect, if possible, any distinguishing chemical properties between these two principles, and further to find out whether any characteristic chemical changes occurred in the samples, with increasing concentration of the active principles, which might be useful in following from a chemical standpoint further attempts at their isolation. As a preliminary attack along these lines the sulfur, nitrogen, cystine, tyrosine, arginine, and histidine contents of the various fractions were determined. A striking difference was found in the cystine content of highly purified preparations of the pitressin and pitocin as determined by the Sullivan method. For example, a sample of pitocin possessing 500 units of oxytocic activity per mg. contained 3.06 per cent sulfur and had a cystine value of 8.96 per cent, whereas a
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sample of pitressin containing 200 units of pressor activity per mg. gave only a faint Sullivan reaction, although 3.10 per cent sulfur was present. In the case of both series of preparations increasing potency was attended by an increase in sulfur content. Another finding which may be of significance was the high tyrosine or rather phenolic value of both series of fractions which markedly increased upon concentration of the active principles. Of the two samples mentioned above the pitocin contained 14.3 per cent tyrosine, while the pitressin sample contained 10.5 per cent. Further work is being carried out along these lines and in further attempts to purify these active principles.

THE METABOLISM OF \textit{dl}-METHIONINE IN THE RABBIT

\textsc{By Robert W. Virtue and Howard B. Lewis}

(From the Department of Physiological Chemistry, Medical School, University of Michigan, Ann Arbor)

The sulfur of \textit{dl}-methionine, when fed in moderate amounts (equivalent to 400 mg. of sulfur) to rabbits, was readily oxidized, the distribution and recovery of urinary sulfur being approximately the same as when cystine was fed in comparable amounts. After subcutaneous injection of methionine, the sulfur of this amino acid was also oxidized readily. After subcutaneous injection of a methionine derivative in which the \(\alpha\)-amino group was "blocked," benzoylmethionine, no evidence of oxidation of the sulfur present in the compound was obtained. No increases in urinary sulfate sulfur were observed, while the excretion of organic sulfur was greatly increased. These results are similar to those previously obtained with cystine, in which it was shown that after "blocking" the \(\alpha\)-amino group of cystine, oxidation of the sulfur of the derivative by the organism of the rabbit did not occur readily.\(^6\) After oral or parenteral administration of methionine, evidence was obtained which indicated the excretion of a small amount of a compound containing the S—S linkage.

Experiments on hypophysectomized female rats support data by Evans et al. on the dog and rat and of Engle on the monkey that the gonad-stimulating substance of urine from pregnant cases does not act the same as do extracts from the gland.

Twenty-one albino females, weighing 120 to 125 gm., when completely hypophysectomized failed to show ovarian stimulation comparable to the normal when injected with anterior pituitary-like substance 2 to 47 days after hypophysectomy. The doses ranged from 4 to 330 rat units injected subcutaneously daily for 3 to 15 days, with total doses of 12 to 1800 rat units. This is in agreement with Evans et al. but contrary to the report by Freud. Although no follicular growth could be detected, some tendency to cause luteinization of the theca and granulosa could be found. This is in partial agreement with the findings of Collip and co-workers.

Four hypophysectomized animals showed some ovarian stimulation after treatment with anterior pituitary-like extracts. However, in three of these the control ovary removed before treatment was begun presented a picture atypical of complete hypophysectomy. Serial sections of the sella turcica failed to reveal any pituitary tissue. The response in these cases was much less than in normal or definitely incompletely hypophysectomized animals. A different explanation is sought. In several normal and incompletely hypophysectomized animals the injection of anterior pituitary-like extracts caused definite follicular growth and formation of corpora lutea. Luteinization of follicles and theca was

In some cases ovulation had not taken place before luteinization. All animals whether showing ovarian stimulation or not responded with cornified vaginal smears. This is in agreement with Collip et al., as well as Freud but is not taken as an index of response since spayed hypophysectomized animals injected with comparable doses of the extract showed some cornification of the vaginal smear, although less than those with intact ovaries. This last was not found by Collip et al. with placental extracts.

Preliminary work on injecting hypophysectomized females with the hypophyseal growth hormone prepared by the method of van Dyke and Wallen-Lawrence74 plus the gonad-stimulating extract of urine indicates that the ovarian response is greater than with the latter alone. This might be expected on the basis of the work by Evans et al.

Likewise, preliminary work with the hypophyseal sex factor prepared by a slight modification of the method of Wallen-Lawrence and van Dyke75 indicates that follicular growth may be produced in hypophysectomized animals.

THE FRACTIONATION OF THE GONAD-STIMULATING SUBSTANCE OF THE ANTERIOR LOBE OF THE PITUITARY BODY

By ZONJA WALLEN-LAWRENCE

(From the Department of Physiological Chemistry and Pharmacology, the University of Chicago, Chicago)

Preparations which have heretofore been referred to as the "gonad-stimulating substance" of the anterior pituitary body have recently been separated into what appears to be two fractions which can be differentiated physiologically. The gonad-stimulating Preparation A, prepared from whole sheep pituitary gland powder by the method described previously for making pressor-free extracts and powders, was reprecipitated once, giving Powder B which formed the starting point for the separation here considered. By using suitable variations in pH, in temperature, and in concen-

As in previous work reported from this laboratory, these preparations were assayed in immature albino rats; injections were given once daily for 4 days beginning on the 21st day of life. Necropsies were performed on the 6th day after the first injection.

In doses adequate to produce significant increases in ovarian weights, Fraction C almost invariably caused the appearance of hemorrhagic follicles in the ovaries. It caused significant but seldom marked uterine hypertrophy or distension. It rarely caused opening of the vaginal introitus and it frequently produced estrus by the 26th day of life. These statements are based on observations in only 52 rats. Fraction C did not differ significantly from Powder B from which it was prepared.

Fraction D, on the other hand, differed from Powder B and Fraction C. In doses sufficient to produce excessive ovarian hypertrophy, Fraction D caused a very striking uterine hypertrophy accompanied by opening of the vaginal introitus and estrus on the 26th day of age. The ovaries contained ripe follicles but no hemorrhagic ones. In doses adequate to produce significant but not excessive ovarian hypertrophy, Fraction D again caused uterine hyperemia, hypertrophy accompanied by estrus, and ripening of follicles without follicular hemorrhage. In doses inadequate to produce significant increase in ovarian weights, opening of the vaginal introitus, uterine hyperemia, hypertrophy and distension, and estrus nevertheless were observed. Even in those few instances in which the ovaries of the injected animals weighed less than their litter mate controls the uterine weights were increased more than 100 per cent and the animals were in estrus on the 26th day of life. The effect of this preparation in ovariectomized rats is being studied.

The effects of Powder B and its derivatives Fractions C and D have also been studied in male rats. The results indicate that the male sex organs are significantly increased in weight even though only four injections were given and necropsies were performed on the 6th day after the first injection. The data show that the testes are as sensitive indicators of anterior pituitary stimulation as are the ovaries of litter mates. This statement is contrary to what has been recorded previously for pituitary implants and for cruder pituitary extracts.
Fractions C and especially D are unstable when kept as dry powders or in aqueous solution under conditions which, in cruder preparations, cause no significant deterioration in months.

The histological studies of the tissues stimulated by these preparations are under way.

A METABOLIC STUDY OF A CASE OF LORAIN TYPE OF INFANTILISM

By CHI CHE WANG, CORINNE HOGDEN, MILDRED KAUCHER, AND MARY WING

(From the Children’s Hospital Research Foundation and the Department of Pediatrics, College of Medicine, University of Cincinnati, Cincinnati)

This paper covers a part of a metabolic study conducted on a 10 year old dwarf boy weighing 15.4 kilos, measuring 104.4 cm. on admission, and belonging to the class of Lorain type of infantilism. Physically he looked like a child of 4 or 5, but mentally he was up to his age. According to his mother he ceased to grow at the age of 3.

Three series of experiments of 14 days each were made on the child. During the first period he was allowed to choose both the kind and the quantity of foods and he received on the average 3.55 gm. of protein and 91 calories per kilo per 24 hours. The protein of the second period was increased to 4.65 gm., while that of the third period was decreased to 2.65 gm. per kilo; otherwise these two diets were not much different from the first. During the last 6 days of each period specimens of foods and excretions were collected and analyzed for nitrogen, energy value, phosphorus, calcium, sodium, magnesiu, and fat. Basal metabolism and creatine and creatinine in urine were also determined during each period. This report gives the first three and basal metabolism only.

Basal metabolism of the three periods showed no difference with an average for sixteen tests of 748 calories per 24 hours, which is from 17.0 to 35.2 per cent below any normal standards for his age. In agreement with results obtained from adult dwarfs by previous investigators, however, his metabolism was within normal limits when it is compared with that of children of his height or when it is expressed on the basis of body surface.

Unexpectedly his protein and mineral metabolism showed a typical resemblance to that of a rapidly growing child. His retention of nitrogen for the three periods averaged respectively 0.082,
0.083, and 0.086 gm. per kilo per 24 hours as compared with 0.041, 0.084, and 0.039 gm. obtained from a group of normal children of his age receiving similar diets. The corresponding values for phosphorus were 0.002, 0.005, and 0.008 gm. as against 0.009 to 0.036 gm., which is the range for normal children. His phosphorus retention was, therefore, slightly below normal.

Although his caloric intake per 24 hours was about 30 per cent below that of a child of his age, his average values per kilo per 24 hours, being 89, 94, and 78 calories respectively for the three periods, were much higher than that of a group of normal children receiving a normal diet, 74 calories per kilo. The high caloric intake during the first two periods was accompanied by a high energy expenditure for activity. The energy expenditure on low protein diet was slightly lower than that of normal children on a normal diet.

One of the most interesting findings in this study is the rate of growth of the child during the 51 days he was in the metabolism unit. He gained 2.9 kilos in weight and 2.1 cm. in height on a wholesome diet with no medication of any kind. A normal boy of his age should gain 0.317 kilo and 0.7 cm. in the same period. His growth in weight was, therefore, 791 per cent and that in height 200 per cent higher than normal. Since his discharge from the hospital he has been back twice for physical examinations. Each time he was found to be losing weight. On February 3, 1933, his weight was 16.0 kilos as compared with 18.3 on May 11, 1932. His height remained unchanged during the 9 months.

THE MECHANISM OF THE ACTION OF METHYLENE BLUE AND SODIUM NITRITE IN CYANIDE POISONING

By WILLIAM B. WENDEL

(From the Laboratory of Biological Chemistry, Washington University School of Medicine, St. Louis)

The experiments of Sahlin,76 Eddy,77 Brooks,78 Hug,79 and Geiger80 demonstrate that methylene blue antagonizes cyanide

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79 Hug, E., Compt. rend. Soc. biol., 111, 519 (1932).
poisoning. Hug finds sodium nitrite more efficacious than methylene blue.

Sahlin and Brooks interpret the phenomenon (with methylene blue) as being analogous to the effect of the dye in overcoming cyanide inhibition of tissue respiration in vitro; that is, the dye acts in lieu of the tissue respiratory catalysts, the activity of which is suppressed by cyanide. A simpler explanation follows from the known behavior of the system methylene blue-hemoglobin-HCN in vitro, namely, the oxidation of hemoglobin to methemoglobin by the dye and the fixing of HCN by methemoglobin. Furthermore, it is known that methylene blue causes the formation of methemoglobin in vivo also.

That this is the explanation is demonstrated by the following illustrative experiments. Removal from a dog of 20 cc. of blood (equivalent to 0.2 mM O₂ capacity) per kilo of body weight and reinjection of the cells suspended in salt solution, after converting the hemoglobin to methemoglobin by amyl nitrite (the excess being removed by washing), make the animal insensitive to immediate intravenous injection (during 2 minutes) of 3 mg. of HCN (0.11 mM) per kilo, a fatal subcutaneous dose. (Five control animals died in 20 to 26 minutes.) Or, injection of the same volume of methemoglobin cell suspension, beginning 13 minutes after subcutaneous injection of 3 mg. of HCN per kilo, revived the animal. During the injection, which lasted 5 minutes, gasping ceased and struggling was resumed. 10 minutes after beginning the injection the animal walked about, and seemed almost normal.

From the fact that the antidotal action of methylene blue and nitrite involves conversion of oxygen-carrying blood pigment into non-functional pigment, it follows that the successful use of these substances is limited by the extent to which this transformation can be tolerated. Whether methylene blue acts also to replace cellular catalysts, in vivo, cannot be demonstrated because of this limitation.

82 Combemale, M., Compt. rend. Soc. biol., 43, 300 (1891).
A DIETARY FACTOR CONCERNED WITH CARBOHYDRATE METABOLISM

BY LAURENCE G. WESSON AND F. C. MURRELL

(From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville)

A metabolic abnormality in rats in which carbohydrate is converted into fat to a degree not found in normal animals is apparently caused by a deficiency in their restricted diet of some factor occurring in certain fats, among them lard. The liquid fat fraction of lard (alcohol crystallization) is, on the average, effective in one-tenth the amount of the solid fat fraction which is necessary to restore partially or completely the course of carbohydrate metabolism to normal. Moreover, a more nearly normal metabolism following this return to normal is maintained without further dosing for a longer period of time with the liquid fat fraction than with the solid fat fraction (25 days as compared with 18 days), although 10 times the amount of the latter is used. The saponifiable fraction of lard is also active in restoring the carbohydrate metabolism to normal, while purified ethyl stearate, which is used as a control substance, is inactive.

The feeding of 6.7 mg. daily of either the liquid or solid fractions of lard to young rats for a period of 4 months practically eliminates the appearance of the metabolic abnormality, while litter mates receiving no fat show abnormal metabolism.

DETERMINATION OF THE HYDROXYL NUMBERS (ACETYL VALUES) OF OILS, FATS, AND WAXES

BY EDWARD S. WEST, GEORGE H. CURTIS, AND CHARLES L. HOAGLAND

(From the Laboratory of Biological Chemistry, Washington University School of Medicine, St. Louis)

A simplified procedure has been worked out based upon the reaction of acetic anhydride + pyridine with the hydroxyl groups. A sample of material is weighed into a special distillation apparatus.
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tus to which is added an accurately measured quantity of a mixture of acetic anhydride and pyridine. The apparatus is heated in a bath at 140–145° with a stream of nitrogen passing through. The pyridine and unused anhydride distil off and are received in absorption flasks containing standard alkali. The alkali used is determined by back titration with standard acid. This, subtracted from the titration value of the original acetic anhydride-pyridine mixture, gives the amount of acetyl bound by the sample, from which the acetyl or hydroxyl number may be calculated.

AN INVESTIGATION OF A NEW METHOD FOR PRODUCING A DIET DEFICIENT IN CYSTINE AND METHIONINE

BY ABRAHAM WHITE AND RICHARD W. JACKSON

(From the Department of Physiological Chemistry, Yale University, New Haven)

Growth studies on the albino rat have been conducted with the object of obtaining a dependable and easily prepared diet deficient in the known sulfur-containing amino acids. In view of the fact that the rat, like the dog and the rabbit, is known to conjugate cystine with bromobenzene and to excrete the resulting bromophenylmercapturic acid in the urine, it seemed promising to investigate the value of bromobenzene as an agent for depleting the animal of a significant portion of its cystine resources. Jackson and Block84,85 have shown that the rat ingesting a type of diet previously designated as cystine-poor would respond in growth to the administration of methionine. The aforementioned authors concluded either that both cystine and methionine are indispensable in the usual sense, or, more probably, that there exists some measure of metabolic interconvertibility between the two amino acids. Furthermore, White and Lewis86 have demonstrated the similarity of the changes in sulfur excretion following the administration of either cystine or methionine with bromobenzene to the dog, and have suggested that methionine may function as does cystine in the detoxication of the monohalogen derivatives of benzene. These findings provided the background for the working hypothesis that the incorporation of bromobenzene in the diet might result in a

84 Jackson, R. W., and Block, R. J., Science, 74, 414 (1931).
deficiency of both cystine and methionine. The general type of experiment planned was obviously analogous to that employed by Griffith\(^8\) to produce restricted growth in the rat by depleting the animal of glycine through the administration of suitable amounts of sodium benzoate.

In our preliminary experiments we have employed animals which, ingesting essentially the basal low cystine-methionine diet employed by Jackson and Block,\(^8\) were found to grow more rapidly than desired (average daily growth of 0.6 gm.). Following the addition of 0.30 cc. (0.45 gm.) of bromobenzene to each 100 gm. of the basal diet, the animals lost weight (average daily decline of 0.6 gm.). The superimposition of L-cystine (0.12 gm.) on the basal diet (100 gm.) already containing the bromobenzene led to definite stimulation of growth (average daily increment of 0.8 gm.). It is to be emphasized that the inflections of the growth curves were immediate and sharp, and strikingly paralleled the dietary alterations. These results were uniformly obtained throughout a group of nine animals observed during experimental periods of 2 to 3 weeks. Moreover, we have indications that under these experimental conditions methionine produces the same type of growth response.

Further experiments have been initiated in the direction of simplifying the basal diet to be employed with the bromobenzene. The particular basal diet now under consideration is one containing 8 to 10 per cent of casein, with an abundant supplement of the vitamin B factors in the form of dried yeast. This diet with a suitable addition of bromobenzene would appear to offer promise in the development of a readily available and reliable procedure for the investigation of the metabolism of cystine and methionine and allied problems by the growth method.

**THE SYNTHESIS OF GLYCYLTAURINE AND GLYCYLECYSTEIC ACID**

**BY JULIUS WHITE**\(^*\)

*(From the Department of Physiological Chemistry, Medical School, University of Michigan, Ann Arbor)*

Glycylecysteic acid was prepared by the oxidation of glycylcysteine by bromine, but could not be obtained by the action of chloroacetyl chloride on cysteic acid and subsequent replacement of


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the chlorine by ammonia. This seems to indicate that in consequence of the accumulation of "negative groups" as a result of the oxidation of the disulfide to a sulfonic acid group, the reactivity or basicity of the amino group may be decreased. Glycyltaurine was prepared readily by the action of chloroacetyl chloride on taurine and replacement of the chlorine by ammonia. This difference in behavior of cysteic acid and taurine may be explained by the fact that taurine has one less negative group (carboxyl group) than cysteic acid and hence the amino group is more reactive. The biological behavior of these "peptides" is under investigation.

THE QUANTITATIVE SPECTROGRAPHIC DETERMINATION OF INORGANIC BASES IN BIOLOGICAL MATERIAL

BY FRANK H. WILEY, J. S. OWENS, AND O. S. DUFFENDACK
(From the Department of Internal Medicine, Medical School, and the Department of Physics, University of Michigan, Ann Arbor)

By use of solutions in which the ratios of the concentrations of sodium, potassium, calcium, and magnesium are approximately those found in urine ash, an attempt has been made to devise a method for spectrographic analysis.

A primary current of 9 amperes and 120 volts was used in a transformer whose secondary coil delivered about 25,000 volts to the spark gap. A vertical spark was formed between a silver electrode at the top and a lower electrode formed by allowing the solution subjected to analysis to flow around a platinum wire inclosed in a small quartz tube. Only the lower half of the spark was photographed.

The solutions were made up to contain 1 per cent of cadmium, in the form of the chloride, to act as the standard. The blackening of the lines on the photographic plate was determined with a microphotometer. By means of a calibration pattern placed on each plate by substituting a step diaphragm for the slit on the spectrograph and by using a spark between aluminum electrodes in a hydrogen atmosphere as the source of light, the intensity of each wave-length of light could be determined from the blackening of the corresponding line on the plate by the method described by Duffendack and Wolfe.88

Calibration curves were made, with solutions of known concen-

tration, in which the concentration of the element to be determined is plotted against the ratio of the intensity of the light emanating from that element to the intensity of the light due to excitation of the cadmium. By plotting similar ratios found in solutions of unknown composition, the concentration of the elements may be determined.

Preliminary results indicate that analyses as accurate, and in some cases more accurate, than those obtainable by chemical methods can be made in a fraction of the time usually required.

THE DETERMINATION OF ANSERINE IN MUSCLE

By D. WRIGHT WILSON and WILLIAM A. WOLFF

(From the Department of Physiological Chemistry, School of Medicine, University of Pennsylvania, Philadelphia)

1 nitrogen atom of anserine or of carnosine reacts with nitrous acid quantitatively in the time required for $\alpha$-amino acids. 2 nitrogen atoms react after hydrolysis of either compound. With this information, a quantitative method for the determination of anserine in muscle has been devised. Fresh, hashed muscle is extracted with water at $70^\circ$ and the slightly acid extract is heated to boiling. The filtrate after concentration is treated with 6 volumes of alcohol, a small precipitate of protein removed, and the solution treated with mercuric sulfate and alcohol until precipitation is complete. After decomposing the precipitate with $\text{H}_2\text{S}_2$, and washing the mercuric sulfide, the neutralized solution is concentrated on a water bath with a fan. The carnosine is determined by the colorimetric method of Koessler and Hanke. Amino nitrogen is determined by Van Slyke's procedure, before and after hydrolysis with hydrochloric acid. The increase of amino nitrogen on hydrolysis is due almost entirely to carnosine and anserine. By subtracting from the total the amount of carnosine determined colorimetrically, the anserine content of muscle is obtained.

A simpler but less accurate procedure involves the use of the colorimetric and gasometric methods with a muscle extract which has been treated with lead acetate only.
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