BEHAVIOR OF SUDAN III IN THE ANIMAL ORGANISM.

By B. E. READ.

(From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven.)

(Received for publication, November 18, 1918.)

The fat-soluble dye Sudan III, since its first preparation by Nietzki in 1880, has been used extensively in the study of problems directly and indirectly associated with vital staining and the metabolism of fat. The work of Daddi, Cage, Riddle, Mendel, Whitehead, and many others, including that recently published by Hatai, has shown how fat can be traced in the body by means of oil-soluble dyes.

In their paper on the behavior of fat-soluble dyes in the animal organism, Mendel and Daniels discussed the toxicity, absorption, transport, and excretion of this dye. They stated that it is apparently non-toxic, inasmuch as animals fed over long periods of time showed no deleterious results. When fed in solution in fat it may enter the organism from the alimentary tract through the lymphatics or by the portal circulation dissolved in reabsorbed bile, a cycle becoming established between the intestine, bile, and blood. The dye is eliminated in the feces alone, except when an alimentary lipuria arises; then it may occur in the urine. Moreover, it is deposited in the adipose tissues and bone marrow, stained fat being no less available to the organism than unstained.

These findings were later discussed by Salant and Bengis in their physiological and pharmacological studies of fat-soluble dyes. Using exceedingly large doses, they found that Sudan III was not pronouncedly toxic. Administered intraperitoneally, subcutaneously, intravenously, or by mouth the dye was reported

1 Nietzki, R., Ber. chem. Ges., 1880, xiii, 800.
4 Salant, W., and Bengis, R., J. Biol. Chem., 1916, xxvii, 403.
to be excreted by the kidney, sometimes found in the bile, and deposited in the adipose tissue only. When observed a few hours after experiment, the central nervous system sometimes showed faint traces of color. Salant and Bengis contended that the absence of dye in the urine as reported by Mendel and Daniels was due to the abnormality of the animals, because only in the case of diseased animals did Salant and Bengis find absence of dye in the urine. This outcome they ascribe to decreased permeability of the kidneys.

The recent work of Hatai on the several effects of feeding Sudan III to rats may well be subjected to criticism and maybe to revision in the light of the results reported below. He found that the feeding of Sudan III to young rats retarded the growth of the thymus, testes, and ovaries. Postmortem examination showed no trace of dye in the brain, spleen, or heart; but its presence was noted in the liver, pancreas, and kidney.

The original purpose of the present experiments was to reinvestigate the seemingly contradictory statements with regard to the path of elimination of the fat-soluble dye Sudan III, and particularly the contention of Salant and Bengis regarding the excretion of Sudan III by the kidneys. The products of various manufacturers were used and gave varying results, which led to interesting observations on the doubtful purity of preparations of Sudan III obtainable on the market at present. Experiments made with a purified product, on the other hand, gave definite results, thus showing the need for careful examination of all preparations of Sudan III used in scientific work to guarantee its purity before drawing conclusions supposedly based upon its properties as a pure, neutral fat-soluble dye.

Properties of Sudan III.

There are many references in the literature to the preparation and properties of Sudan III, which may be briefly summarized as follows:

[Sudan III, C_{21}H_{16}N_4O. Synonyms.]

Benzeneazobenzeneazo-β-naphthol, C_{15}H_{16}N = N - C_{3}H_{4} - N = N - C_{10}H_{6}OH. Tetrazobenzol-β-naphthol, Oil Red O, Cerasinrot, Scharlach R. Fettponceau, Motirot 2R, etc. (Some are the methylated compounds.)

---

5 Merck's 1907 Index, New York, 3rd edition, 1907, 429.
It is a brick-red powder, insoluble in water, alkali, or dilute acid; slightly soluble in alcohol; very readily soluble in hot glacial acetic acid, chloroform, ether, paraffin, benzene, oils and fats, fatty acids, and all lipoid solvents. It may be crystallized out of glacial acetic acid in sheeny metal green-brown plates, melting at 195°C.

When treated with strong sulfuric acid it gives a green color. It splits into amino-naphthol, aniline, and p-phenylenediamine when treated with tin and hydrochloric acid. The sodium salt of the sulfonated compound is known as B Scharlach, a much used water-soluble dye.

The peculiar solubilities of Sudan III have facilitated the study of fat digestion and absorption through the intestinal walls. It is even more soluble in bile than in fat, but the question of its solubility in soaps or glycerol does not seem clear. Pflüger states that the pure dye is freely soluble in bile and dissolves in both soap and glycerol giving a deep red solution. Mendel, while reaffirming its solubility in bile even in preference to oil, states on the contrary that it is insoluble in either glycerol or soap solution.

Bearing in mind the above properties we now turn to the examination of products on the market which have been in common use. The samples taken were those put out by (1) Kahlbaum, (2) Berlin Anilin Fabrik, (3) Grüber, (4) A. H. Thomas Co.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Melting point °C</th>
<th>Color</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>130</td>
<td>Brick-red powder.</td>
<td>Very readily soluble in oil.</td>
</tr>
<tr>
<td>2</td>
<td>120</td>
<td>Dark venous-red.</td>
<td>Solution in oil dark colored.</td>
</tr>
<tr>
<td>3</td>
<td>135</td>
<td>Dark brick-red, lumps showing lighter material inside.</td>
<td>Very readily soluble in oil.</td>
</tr>
<tr>
<td>4</td>
<td>120</td>
<td>Orange-red powder.</td>
<td>Solution in oil of deep orange color, more or less soluble in dilute alkalies and acids.</td>
</tr>
<tr>
<td>5</td>
<td>195</td>
<td>Green-brown sheeny plates.</td>
<td>Purified product.</td>
</tr>
</tbody>
</table>


These varying properties show unmistakably the presence of impurities in the commercial products.

**Purification of Sudan III.**

The dye may be separated from its impurities by first dissolving in alcohol or glacial acetic acid, and subsequently precipitating in water to remove all water-soluble substances; the repetition of this procedure results in a fine brick-red powder of low melting point similar to that of the commercial article. It should then be recrystallized out of hot glacial acetic acid three or more times, the purity of the fine sheeny flocculent crystals thus obtained being judged by the melting point. Very impure products need to be dissolved in a lipoid solvent and shaken out with dilute aqueous alkali and acid alternately until the aqueous solvent ceases to take up any further color.

The solubility of Sudan III in oil has been the subject of long discussion. Michaelis\(^8\) considered that it was readily soluble in oil, but does not state to what degree; on the other hand Mann\(^9\) considered the solution to depend on a chemical and not a physical process. Mann concludes from Michaelis' own work that the union between Sudan III and oleic acid is a chemical one depending on the oxidation of the unsaturated fatty compound, analogous to the action of osmium tetroxide, the only difference being that azo dyes form additive compounds with the fat without changing their color, while osmium compounds are readily decomposed.

The chemistry of Sudan III has been worked out in the elaborate researches of Michaelis. He showed that its staining power was dependent on the simple azo group "benzene azobetanaphthol," and varied according to the nature of the rest of the molecule.

Bell\(^10\) in his experiments on the interstitial granules of striated muscle secured his best results with Herxheimer's stain, and relative to the experiments here reported it should be noted that he states, "very variable results were obtained," indicating that the

---

\(^8\) Michaelis, L., *Virchows Arch. path. Anat.*, 1901, clxiv, 263.

\(^9\) Mann, G., Physiological histology, Oxford, 1902, 306.

quality of the dye varied, although "only Grübler's dyes have been used." The methyl and ethyl compounds of benzeneazo-naphthol have a greater staining power—especially when no salt-forming groups are present. The "indifferent" character of Sudan III makes it a peculiarly efficient fat-soluble dye. It has great ability to unite with fat cells in both an acid and alkaline medium as compared with other dyes. This is shown by Robertson\(^{11}\) in his work on tissue staining.

The solubility of Sudan III in oil has an important bearing on the relative availability of stained and unstained fat in the animal body. The assimilation of a solution of the dye would be expected to follow a different path from that of the assimilation of an oil compound, the latter possibly rendering the fat unavailable for use in the organism.

Unlike Scharlach R, Sudan III dissolved in alcohol and mixed with five to six volumes of water does not form a colloidal solution, and apparently does not show the same adsorption phenomena as do some other indifferent dyes. This property should be considered in the interpretation of the supposed urinary excretion of Sudan III.

Sudan III is easily detectable in the body fluids. Simple solution of the dye in alcohol gives a distinct pink color up to one part in ten million; likewise in any of the lipoid solvents when it is present in traces (one part in a million). Gasoline, in preference to ether or toluene, was found to be the cheapest and best solvent. The writer did not find Salant's\(^{4}\) wool method satisfactory for dark colored urines. The wool adsorbs so much of the urinary pigment that it is almost impossible to tell whether there is any dye present. Sudan III is best extracted from lymph by a mixture of one volume of alcohol and two volumes of ether, a quantitative yield being obtained by shaking the mixture and standing it aside for 24 hours.

Absorption and Transport.

Mottram\(^{12}\) showed clearly that animals possess varying degrees of ability to absorb and use fat; therefore one might expect

\(^{11}\) Robertson, T. B., J. Biol. Chem., 1908, iv, 1.

\(^{12}\) Mottram, V. H., J. Physiol., 1915-16, 1, 380.
to find some variation in the results obtained by workers using different animals. Mendel worked chiefly with dog, cats, and rats; Salant used rabbits, the normal diet of which includes very little fat. In one of the following experiments the writer injected intraperitoneally an aqueous suspension of the dye into a rabbit and observed no excretion either in the urine or feces. The animal when killed showed a pink omentum, unstained below the surface, with scattered lumps of dye embedded in it. Intraperitoneal injection of the dye dissolved in oil gave good absorption and excretion, the dye appearing in the feces in 1 to 5 days. Rats injected intraperitoneally showed little absorption of the stained oil. The rats were killed after 2 or 3 weeks and there was found still present in the peritoneum a considerable amount of stained oil. Mendel\(^3\) has shown beyond question that when Sudan III is fed by mouth it passes readily into the lymphatics and thence into the blood stream; later it is eliminated with the bile into the alimentary tract.

There are other factors which influence the nature and rate of absorption of fat and therefore of stained fat. Various adjuncts have been used to assist in emulsifying the oil administered. Mendel used lecithin, a substance which Loevenhart and Souder\(^3\) showed to be an accelerator to the digestive action of pancreatic juice on oils. Salant\(^4\) used emulsions containing sodium carbonate, which would greatly decrease the solubility of the dye in the oil and so probably retard its absorption. Salant also used suspensions in gelatin, which might or might not be absorbed depending on the condition of the animal, such a preparation being comparable to a watery suspension.

In view of the above facts regarding the doubtful absolute purity of some of the preparations of Sudan III heretofore used, the question of the absorption of fat through the intestinal mucosa might well be brought forward again. As the dye has been relied upon as an exact index of the paths of absorption and transport of fat, any small amount of a foreign coloring matter present may give very misleading results, from which erroneous conclusions may have been drawn.

B. E. Read

Excretion.

The work of Abel and Rowntree\textsuperscript{14} on the excretion of dyes leads one to expect Sudan III, on account of its indifferent character, to be excreted in the feces. This was found to be the case by Mendel\textsuperscript{9} and was confirmed in the present work on dogs, all of which were tested by the phenolsulfonephthalein test and found normal in respect to kidney function. Salant claimed that the dye was excreted in the urine, but obtained varying results from animals with deranged kidney mechanism, his experiments being conducted with rabbits to which very large doses of dye were given. The writer repeated this work with rabbits and found variation according to the purity of the dye, the size of the dose, and the kind of diet. In all cases \textit{when the pure dye was fed, even in large amount}, no dye was excreted in the urine, soft deep red stools being obtained in 2 to 4 hours.

Small doses of the unpurified product given intraperitoneally did not appear in the urine. It was impossible to give to rabbits such large amounts intraperitoneally as by mouth. Large doses of the crude drug—as much as 2 gm. at one time—were administered with the aid of a stomach tube to full grown rabbits. During one or more days following, dark reddish brown urines were obtained. The dark red-brown pigment was not Sudan III. It was thought to be probably a foreign pigment present in the original impure dye, soluble in the body fluids, and excretable by the kidney in some form or other. This should not be interpreted as characteristic of the excretion of Sudan III. Pure Sudan III so administered to rabbits did not give pink urine; and the same negative outcome was observed when dogs were given moderate doses of the impure products with their daily food. The colored urines were only obtained when abnormally large doses of the crude dye with its impurities were administered to rabbits. The urines on standing deposited the pigment as a dark brown precipitate. The amount of the pigment seemed much increased when the rabbits were given exclusively an oat diet. Such a diet with its resulting acidosis changes the nature of the renal excre-

\textsuperscript{14} Abel, J. J., and Rowntree, L. G., \textit{J. Pharm. and Exp. Therap.}, 1909-10, i, 231.
tion, and herein may lie the explanation of the appearance of the pigment impurity in the urine.

The pure dye is excreted in the feces only, the earliest appearance observed being after 2 hours. It is extractable by gasoline, whereby it may be estimated quantitatively.

Toxicity.

Weyl\textsuperscript{15} some years ago announced that diazo colors in general are non-poisonous. This has been confirmed in particular with regard to Sudan III by subsequent workers. Mendel stated Sudan III to be harmless even in large doses provided the dye was pure. Salant and Bengis in their experiments observed death after 1 to 3 weeks; but they regarded it as extremely doubtful whether death was due to the dye. Hatai reported that Sudan III retarded to a considerable extent the body growth of albino rats, and caused a striking change in weight of many of the organs, especially the liver, pancreas, thymus, testes, and ovaries.

The following experiments show that the impure dye may be markedly toxic. When 2 gm. were given at one time to normal rabbits, in all cases death ensued in a very short time. A neuritic-like condition of the hind legs developed first, and sooner or later the animals died in convulsions. Rabbits fed on an exclusive oat diet showed paralytic symptoms very rapidly after feeding the dye. The pure dye given in normal amount did not show any of these symptoms.

A number of albino rats were used to test further the toxicity of the various products. Those fed the crude preparations became very much debilitated. The orange colored dye (No. 4) stunted the growth, and brought about a general appearance of malnutrition; and in other rats the hind legs were paralyzed on one or both sides.

Path of Absorption and Excretion of Sudan III in Animals with Normal Kidney Mechanism.

Dogs were fed moderate doses of Sudan III in fatty food, and after several hours cannulas were inserted in the thoracic and bile

\textsuperscript{15} Weyl, T., Die Teerfarben, Berlin, 1889.
ducts to collect the lymph and the bile. Blood was taken from the femoral artery. Just before death the bladder was emptied with great care to avoid contamination, and the urine examined for the presence of the dye. All the animals were tested several hours before operation with the phenolsulfonphthalein kidney function test and were found to be normal.

The following results show that the dye administered with fat is well absorbed into the lymphatics, is transported into the blood, and excreted with the bile, and reabsorbed in it from the intestine. It is not excreted by the kidney, but always appears in the feces. Small doses of the crude products did not influence the color of the urine.

**Experiment 1.**—Dog, female, weight 13.5 kilos. Fed 4 gm. of Sudan III Specimen 1 during 1 week, at the same time adding plenty of lard to the diet. Very soft pink feces were excreted. On the 8th day, 3 hours after taking a heavy fat meal containing Sudan III the animal was operated upon to obtain samples of lymph and bile. Both contained a considerable amount of Sudan III. The milky lymph was intensely pink, and the dark bile, dried down with lime and extracted with ether, showed an abundance of Sudan III. The urine showed no trace of red color. At the conclusion of the operation 1 cc. of phenolsulfonphthalein was injected subcutaneously, and after 9 minutes 2 cc. of urine were obtained giving a strong positive reaction.

**Experiment 2.**—Dog, male, weight 14.3 kilos. 1 gm. of Sudan III Specimen 1 was added with 15 gm. of lard to the morning meal of ground biscuit. After 5 hours the animal showed signs of gastric disturbance; there was vomiting with diarrhea and it was several days before the animal appeared normal again. When fully recovered, the animal was given on 3 successive days 1 gm. of purified Sudan III with the morning meal. On the 3rd day, 1 hour after feeding, an operation was conducted to obtain lymph and bile. More than 100 cc. of a very pink lymph were obtained in 2 hours, and the bile collected was saturated with Sudan III. No dye appeared in the urine. The pure dye did not disturb the digestive system so much as the crude products.

**Experiment 3.**—Dog, female, weight 10.5 kilos. 0.5 gm. of pure dye was fed with 10 gm. of lard, and 2 hours later a similar amount was fed in half a pint of cream. After 4 hours the animal was operated upon as in Experiments 1 and 2. The lymph was decidedly pink, and the bile taken direct from the gall bladder when extracted with gasoline showed the presence of a considerable amount of Sudan III. There was no trace of dye in the urine. At the end of the operation 0.5 cc. of phenolsulfonphthalein solution was injected subcutaneously, and after 7 minutes it appeared in the urine showing that the kidneys were functioning well.
Experiment 4.—Dog, female, weight 12.4 kilos. Fed 1 gm. of pure dye, and after 2 days the dose was repeated with half a pint of cream. 1 hour after giving the second dose the dog was operated upon as before. The lymph flow was exceedingly small but quite pink in color. The bile gave a strong red color when shaken up with gasoline. Blood collected from the femoral artery was allowed to clot and the serum extracted with gasoline. It showed unmistakably the presence of the dye. There was no trace of dye in the urine.

Experiment 5.—Dog, male, weight 11.5 kilos. 1.5 gm. of Sudan III Specimen 1 with lard were added to the usual food. After 1½ hours an operation was conducted to obtain specimens of lymph, bile, blood, and urine. All except the urine showed the presence of Sudan III in marked amount.

Experiment 6.—Dog, female, weight 14.5 kilos. 1 gm. of Sudan III Specimen 4 was given with lard, cream, and biscuits. After 2 hours the body fluids were collected as in Experiment 5. The lymph and blood were strongly positive, but the bile was much lighter than usual containing only very faint traces of the dye. The urine, as in all of the experiments, showed no trace of Sudan III.

TABLE I.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Dye</th>
<th>Sudan III in</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>++++</td>
</tr>
<tr>
<td>2</td>
<td>Pure</td>
<td>++++</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>++</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>++</td>
</tr>
</tbody>
</table>

The results with normal animals confirm the findings of Mendel and Daniels, and answer clearly the criticism of Salant and Bengis.

To investigate further the nature of the absorption and excretion of Sudan III, experiments similar to those of Salant and Bengis were conducted on rabbits. The protocols of the various experiments bring out especially the toxicity of the crude products in common use.

Experiment 7. Toxicity of Commercial Sudan III, Specimen 1.—Rabbit 1, female, weight 1.6 kilos. Mixed diet (oats, corn, cabbage, and carrots). 84 mg. were injected intraperitoneally with 5 cc. of oil. During the following 3 days soft, deep red feces were obtained but the urine was nor-
B. E. Read 131

mal. Similarly a week later 126 mg. were given in 10 cc. of oil. 8 days later 2.72 gm. in 20 cc. of oil were given by mouth. Intensely red soft feces were excreted in 3½ hours. Subsequent diarrhea made it difficult to keep the animal clean, the belly and hind quarters becoming smeared very red. The toxic symptoms of the dye developed gradually; after a few hours there was loss of control in the hind limbs and sprawling on the front legs, and the animal died in convulsions within 24 hours. No dye appeared in the urine at any time.

Experiment 8. Effect of Administering Sudan III, Specimen 1, Recrystallized Three Times, Melting Point 130°C., with Exclusive Oat Diet.—Rabbit 2, female, weight 1.76 kilos. The animal was fed on oats for several days, when the daily excretion of urine became very small. 92 mg. of dye were injected with 10 cc. of oil intraperitoneally. In a few days there developed paralysis in the left leg with albuminuria. The sickness continued acute as long as the exclusive oat diet was given. After 25 days carrots were added to the diet, and 2 gm. of the dye were given with 20 cc. of oil. A very heavy, dark brown urine was voided for 6 days, after which time the urine returned to its normal color, and the paralysis in the leg was much improved. The animal died several weeks later. The pigment in the urine was examined for bile pigment with a negative result. It precipitated out on standing. Hydrolyzed with acid and alkali it failed to give any red color. On account of its general properties and the fact that the administration of pure Sudan III did not produce this pigment, the pigment must have originated as an impurity in the commercial specimen of the dye.

Experiment 9. Toxicity of Commercial Sudan III, Specimen 3.—Rabbit 3, female weight 1.7 kilos. Diet of oats and carrots. 2 gm. of the mixed impure residues from the recrystallization of the dye were given with oil by mouth. After 2 hours soft red feces were excreted, which in 4 hours changed to a red liquid stool. The urine collected during the following 6 days was a thick red colloid, after which time the animal appeared normal in every way. 12 days later 2 gm. of commercial Sudan III, Specimen 3, were given with oil by mouth. The animal excreted red-brown urine during several days, and gradually developed neuritic-like symptoms, the right side being first affected, and later the hind legs being completely paralyzed. At the end of the 3rd week the rabbit had a convulsion, and the following day died in convulsions.

Experiment 10. Effect of Administering Impure Sudan III, Specimen 4.—Rabbit 4, female, weight 1.84 kilos. Exclusive oat diet. 2 gm. of the dye suspended in water were given by mouth. On the following day only the feces were colored red, but a very dark brown urine was excreted for a week. After 3 weeks the animal was killed. It showed no stain either in the omentum or any of the fatty tissues of the body. The urine after standing a week deposited the pigment on the sides of the vessel. This brown material was examined as before, and was also apparently an impurity in the original dyestuff. There was degeneration of the liver. It was concluded that the dye failed to enter the circulation at all; otherwise at least the omentum would have been stained.
Experiment II.-Absorption, Distribution, and Excretion of Pure Sudan III Administered to an Animal on a Good Mixed Diet.—Rabbit 5, female, weight 2.0 kilos. Diet of oats, corn, cabbage, and carrots. 1 gm. was given by mouth with 50 cc. of oil. After 3½ hours very red feces were excreted and acute diarrhea followed, lasting several days. The urine was darker than normal, but the natural color returned in 5 days. The animal was killed a month later. The omentum, the fatty tissues of the neck and legs, and the kidney and heart fat all showed distinctly the presence of the dye, being stained pink. The nerves and spinal cord were quite white, and the bile was free from the dye. The pure dye was apparently nontoxic.

Experiment III.—Repetition of Experiment II. Rabbit 6, female, weight 2.1 kilos. Good mixed diet. 1.5 gm. of the purified material were given with 35 cc. of oil by mouth. There was absolutely no sign of any foreign pigment in the urine excreted during the following month. The dye was stored in the omentum and fatty tissues of the body, and excreted in the feces, as in the previous experiment.

Experiment IV.—Effect of Injections of Small Doses of Pure Sudan III.—Rabbit 7, female, weight 1.72 kilos. Diet of oats, corn, and cabbage. 20 mg. of pure Sudan III were dissolved in 5 cc. of peanut oil and injected intraperitoneally. 5 days later 45 mg. in oil were injected subcutaneously, and after 13 days a water suspension of 65 mg. was injected intraperitoneally. There was no immediate appearance of the dye in either the urine or feces. The animal was killed a week later. It showed a heavily stained omentum, the inner layer of which was quite white. The particles of dye from the injection of the watery suspension appeared just scattered over the fatty surface, and were evidently unabsorbed.

Experiments were conducted further with white rats. The toxic effects of the crude impure dyes were most pronounced, being more marked from the preparation with a low melting point and abnormal properties.

Following intraperitoneal injection all specimens caused death in a relatively short time. In each case 200 mg. dissolved in 3 cc. of warm oil were used. If death did not take place the same day the animal was fed on a mixture of the stained oil and dog biscuit. In all cases the abdomen when opened gave off a foul smell. The omentum and intestine both inside and out were loosely coated with the dye. A control experiment conducted with the plain oil proved harmless.

A number of rats after fasting for 4 days were fed specimens of the commercial dye dissolved in oil and mixed with dog biscuit powder. There was produced a general debility and appearance of malnutrition. Specimen 4 was unlike the other crude speci-
TABLE II.

Summary of Rat Experiments with Sudan III.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>3</td>
<td>0.2 gm.</td>
<td>Intraperitoneal.</td>
<td>Died in convulsions.</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>3</td>
<td>0.2 gm.</td>
<td>Intraperitoneal and in food.</td>
<td>Paralysis of hind limbs.</td>
<td>Died after 1 wk.</td>
</tr>
<tr>
<td>16</td>
<td>2</td>
<td>0.2 in 3 cc. oil 0.5</td>
<td>Intraperitoneal. In food.</td>
<td>Very debilitated.</td>
<td>Died after 12 days; empty stomach; stained oil not all absorbed from the peritoneum.</td>
</tr>
<tr>
<td>17</td>
<td>1</td>
<td>0.2 in 3 cc. oil</td>
<td>Intraperitoneal.</td>
<td>“ “</td>
<td>Died in 2 wks.; lost quick movement; hind limbs very slow.</td>
</tr>
<tr>
<td>18</td>
<td>5, pure</td>
<td>0.2 in 3 cc. oil</td>
<td>“ “</td>
<td>Became bad tempered.</td>
<td>Continued to eat well; after 19 days gave Dye 4.</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Ad libitum</td>
<td>In food.</td>
<td>Very debilitated.</td>
<td>Died 3 wks. later; loose hair; granulated ears; foul smelling, stained omentum and kidney fat.</td>
</tr>
<tr>
<td>19</td>
<td></td>
<td>Plain oil 3 cc. 0.2 in 3 cc. oil</td>
<td>Intraperitoneal.</td>
<td>No apparent effect.</td>
<td>After 1 wk. gave Dye 4.</td>
</tr>
<tr>
<td>20</td>
<td>3</td>
<td>Ad libitum</td>
<td>In food.</td>
<td>Debilitated.</td>
<td>Fasted 4 days, fed dye 3 days, then put on normal diet 1 mo., after which Dye 5 was given.</td>
</tr>
</tbody>
</table>
TABLE II—Concluded.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Dye specimen</th>
<th>Dose</th>
<th>Administration of dose</th>
<th>Effect</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>5</td>
<td>0.2 gm. in 3 cc. oil</td>
<td>Intraperitoneal.</td>
<td>Died after 15 days.</td>
<td>First fasted 4 days. During 8 wks. weight almost stationary, varying from 133 to 128 gm.</td>
</tr>
<tr>
<td>21</td>
<td>4</td>
<td>Ad libitum.</td>
<td>In food with oil for 1 wk.</td>
<td>Stunted growth, quite lively.</td>
<td>Same as Experiment 21, varying from 168 to 153 gm. at time of death. Emaciated, very little body fat, no stained fat in the body.</td>
</tr>
<tr>
<td>22</td>
<td>4</td>
<td>&quot; &quot;</td>
<td>&quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>&quot; &quot; &quot; &quot;</td>
<td>Three rats grew normally up to nearly 300 gm. Killed after 8 wks. Omentum, neck, leg, and kidney fat heavily stained.</td>
</tr>
<tr>
<td>23</td>
<td>3</td>
<td>&quot; &quot;</td>
<td>In food for 1 wk.</td>
<td>Debilitated, bad tempered till put back to normal diet.</td>
<td></td>
</tr>
</tbody>
</table>

mens. The rats fed on it were very stunted in growth and did not show staining of the omentum and other fatty tissues of the body.

These experiments with the varied effects on the growth and health of the animal bring out the varied quality of the crude dye products, and confirm the results of the rabbit experiments which show the undoubted toxicity of the unpurified commercial preparations.

SUMMARY.

Sudan III dissolved in oil and administered intraperitoneally, subcutaneously, or by mouth, is absorbed, and may be traced in
the lymph, blood, and bile. It is transported to the fatty tissue of the body, being deposited particularly in the omentum. It is excreted in the feces, and is not found in the urine of normal animals.

Ordinary commercial preparations of Sudan III contain more or less impurity of a toxic nature. They may cause great debility, stunt the growth, produce a neuritic-like condition, and be fatal in large dose. The foreign substances may be excreted by the kidney, and appear in the urine as deep colored pigments.

The purification and properties of Sudan III are set forth, and their important bearing on problems associated with fat metabolism and vital staining discussed.

I am indebted to Professor Lafayette B. Mendel for assistance and suggestions in planning and carrying out the above experiments.
BEHAVIOR OF SUDAN III IN THE ANIMAL ORGANISM
B. E. Read


Access the most updated version of this article at http://www.jbc.org/content/37/1/121.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/37/1/121.citation.full.html#ref-list-1