The Biochemistry of Animal Cells

I. THE EFFECT OF CORTICOSTEROIDS ON LEAKAGE OF ENZYMES FROM DISPERSSED RAT LIVER CELLS

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(Received for publication, November 27, 1962)

In past years there has been great progress in enzyme chemistry, which has contributed to the clarification of many physiological mechanisms in living organisms. Enzymes, however, are not scattered haphazardly in the cell, but are localized in an organized way, often associated with cellular organelles (1). These patterns of enzyme localization control enzyme activities. Typical examples have been shown in mitochondrial respiration coupled with oxidative phosphorylation (2, 3). Results from experiments on subcellular particles should also be true for the intimately related enzymes and cellular structures in whole cells. However, there are few reports on whole cells available. From this point of view, in order to study the behavior of enzymes within the cells, it is desirable to employ dispersed intact cells suspended in a simple, defined medium. Furthermore, the study of the specific functions of organs would also be advanced if investigations were carried out at a cellular level.

There have been a number of reports on procedures suitable for making cell suspensions, with the use of enzymatic digestion (4, 5), filtration through a sieve (6), acid treatment (7), and perfusion of the tissue with chelating agents (8). Cell suspensions obtained by the above procedures, however, have two major disadvantages for use in biochemical studies. One is the low rate of respiration of the resulting cells, and the other is the enzyme leakage from the cells. Laws and Stekland (9) and Kalant and Young (10) reported that such cells had remarkably low endogenous respiration and that succinate was the only substrate which could be metabolized.

Henley et al. (11, 12) found that many enzymes, such as lactic dehydrogenase, malic dehydrogenase, and glutamic-pyruvic transaminase, leaked out from rat liver cells when the cells were dispersed. They showed that the leakage of these enzymes was not prevented by addition of various compounds to the suspending medium.

In order to overcome these obstacles and to obtain cells with normal function, experiments were carried out on the addition of ADP to the medium, and the rate of cellular respiration was found to be restored. The preliminary results of this work have been reported (13).

The present paper shows that in suspension, rat liver cells lose many enzymes, including tryptophan pyrrolase, serine and threonine dehydrases, and two-thirds of their glutamic-pyruvic transaminase activity. The leakage of these enzymes, and particularly of the glutamic-pyruvic transaminase, could be prevented if the rats were treated with various glucocorticoid hormones before the liver cells were dispersed. It is also reported that the anti-inflammatory actions of these hormones are closely related to their actions in preventing enzyme leakage.

EXPERIMENTAL PROCEDURE

Preparation of Cell Suspension and Homogenate—Of the various methods for the preparation of cell suspensions tested, the methods of Anderson (8) and of Branster and Morton (14) were finally adopted for the present work, because these methods yielded relatively large amounts of cells. The procedure used was as follows. Male albino rats of the Donryu strain, weighing 170 to 200 g, were decapitated. The liver was perfused, via the inferior vena cava and the portal vein, with 30 ml of calcium-free Locke's solution, containing 0.027 M sodium citrate, at 37°C. The liver was then removed, washed with Locke's solution, weighed, and divided into two portions.

Four grams of the liver were dispersed as a cell suspension. The homogenizer used for preparing the cell suspension was a siliconized glass test tube with a loosely fitting, smooth surfaced pestle, made of acrylic resin. This homogenizer was similar in size and shape to that used by Kamphausen and Morton (15). The liver was suspended in Locke's solution (5 ml per g of liver) and gently crushed in the homogenizer by hand, with 10 up and down strokes used for the operation. The crude cell suspension thus obtained was filtered through a metallic sieve (200 mesh) and the filtrate was centrifuged at 100 × g for 3 minutes. The resulting supernatant fraction was used, if necessary, for DEAE-cellulose column chromatography as shown in Fig. 4. The precipitate was suspended in 30 ml of Locke's solution by gentle agitation with a glass rod. The suspension was placed in an ice bath for 10 minutes and then centrifuged. The packed cells thus obtained were dispersed in 10 ml of distilled water. At this stage microscopic examination showed negligible contamination with blood cells and cell debris. This suspension of liver cells was immediately homogenized in a Potter-Elvehjem glass homogenizer and then sonically disrupted in a 10-ke sonic oscillator for 5 minutes (cell homogenate). The rest of the liver was homogenized with 10 ml of distilled water in a Potter-Elvehjem glass homogenizer and sonically treated as described above (original homogenate).

Both homogenates contained about 50 mg of material, dry weight, per ml, and were appropriately diluted before use in experiments.

All of the above procedures, except liver perfusion, were carried out at 4°C and pH 7.0.

In order to establish a routine method for the preparation of...
the cell suspension, the time taken for each step was fixed. Thus 15 minutes were allowed for cell filtration; 10 minutes for the first centrifugation; and 25 minutes for washing, allowing the cells to stand, and the second centrifugation. The cells obtained were immediately homogenized, so that the total time taken from crushing the liver until homogenization was complete was 50 minutes.

Assay Methods—For assay of glutamic-pyruvic and glutamicoxaloacetic transaminases, the method of Wada and Snell (16) was slightly modified. The reaction mixture for glutamic-pyruvic transaminase contained potassium phosphate buffer, pH 8.0, 100 µmoles; sodium α-ketoglutarate, 10 µmoles; γ-aminobutyric acid, 10 µmoles; pyridoxal phosphate, 30 µg; and homogenate, 0.1 ml (about 0.5 mg, dry weight) in a total volume of 2 ml. After 5 minutes incubation at 37°C, the reaction was started by addition of the L-alanine. After further incubation for 10 minutes, the reaction was stopped by the addition of 1 ml of 10% metaphosphoric acid. The pyruvate formed was measured by the method of Friedemann and Haugen (17). Xylene was used as solvent, and the xylene layer was washed with 4 ml of 0.5 N HCl and then extracted with sodium carbonate solution (16). For assay of glutamic-oxaloacetic transaminase activity, alanine was replaced by L-aspartic acid in the above system, and a more dilute homogenate (25 to 50 µg, dry weight) was used. The oxaloacetate formed was converted to pyruvate by the aniline-citrate method (16), and the pyruvate was measured as described above.

For assay of serine and threonine dehydrases, the reaction mixture contained potassium phosphate buffer, pH 8.0, 100 µmoles; L-serine or L-threonine, 10 µmoles; pyridoxal phosphate, 30 µg; and homogenate (15 mg, dry weight). The mixture was incubated for 20 minutes. The pyruvate or α-ketobutyrate formed was measured by the method of Friedemann and Haugen's method (17) with the use of ethyl acetate as the extracting solvent for α-ketobutyrate.

Glucose 6-phosphatase, lactate dehydrogenase, and tryptophan pyrrolase were measured by the methods of Cori and Cori (18), Neilands (19), and Knox (20), respectively. The activities of the various enzymes are expressed in millimicromoles per hour per mg of homogenate, dry weight, at 37°C. Because the recovery of cells is variable from one preparation to another, comparison of the total activities of the original homogenate and of the cells is impossible. Therefore the retention of each enzyme, i.e., the amount of enzyme remaining in the dispersed cells, was expressed as a percentage calculated from the ratio of the specific activity of the cell homogenate to that of the original homogenate. This mode of expression may be defended on the basis of findings by Henley et al. that there is not significant general leakage of proteins during preparation of the suspensions (12).

Protein was estimated by the modified Biuret reaction of Pardee.1

Treatment of Rats with Corticosteroids—Corticosteroids were injected intramuscularly into rats daily. The dose and number of injections varied, as described in “Results.” Control rats received injections of 0.9% NaCl. During the injection period, food and water were available ad libitum.

When puromycin was injected into the rats together with dexamethasone, 15 mg of puromycin were injected into the rats first, and after 1 hour the animals received 1 mg of dexamethasone and 15 mg of puromycin. Subsequently 15 mg of puromycin alone were injected four times at hourly intervals, and 1 hour after the last injection the rats were killed. Thus the total amount of puromycin injected was 90 mg.

**RESULTS**

Enzyme Leakage from Cells in Suspension—Microscopic examination of the dispersed cells showed that they were homogeneous parenchymatous cells and were well dispersed (Fig. 1). They also retained their structural organization without any recognizable damage, and there was remarkably little contamination with subcellular constituents or fragments of damaged cells. There are several reports showing that dispersed cells showed no structural damage that was recognizable by electron microscopy (14, 21).

However, when the liver was dispersed as a cell suspension, there was considerable loss of activity of various cellular enzymes (Table I). The enzymes in the soluble fraction especially, such as tryptophan pyrrolase, lactate dehydrogenase, and serine and threonine dehydrases, were almost completely lost from the cells. On the other hand, glucose 6-phosphatase, known to be localized in microsomes (22), stayed entirely in the cells. Our results also

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1. A. B. Pardee, personal communication.
2. 1α-Methyl-4α-fluoro-1-dehydrocortisol.

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**Fig. 1.** Phase contrast micrograph of dispersed rat liver cells. Magnification, X100.
The leakage of these enzymes was not prevented. Henley, Sorensen, oxidizable substrates. These facts suggest that enzyme leakage from dispersed cells may not be energy-dependent. Therefore it

phenol, to the medium used for perfusion and dispersion. In addition, even when enzymatic methods were used for dispersion of the cells (23). These substances included ATP, hemoglobin, and serum albumin. We also observed that enzyme leakage was not prevented by addition of ATP together with oxidizable substrates such as succinate or fumarate, or of dinitrophenol, to the medium used for perfusion and dispersion. In addition, when the cell suspension was prepared at 37°C, enzyme leakage was not prevented in the presence or absence of oxidizable substrates. These facts suggest that enzyme leakage from dispersed cells may not be energy-dependent. Therefore it

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>No. of samples</th>
<th>Enzyme activity</th>
<th>Retention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate dehydrogenase</td>
<td>4</td>
<td>2,280 ± 90³</td>
<td>120 ± 15 ³</td>
</tr>
<tr>
<td>Serine dehydrogenase</td>
<td>5</td>
<td>54 ± 1.8</td>
<td>0</td>
</tr>
<tr>
<td>Threonine dehydrogenase</td>
<td>5</td>
<td>42 ± 4.9</td>
<td>0</td>
</tr>
<tr>
<td>Tryptophan pyrrolase</td>
<td>4</td>
<td>2.2 ± 0.5</td>
<td>0</td>
</tr>
<tr>
<td>Glutamic-pyruvic transaminase</td>
<td>10</td>
<td>1,690 ± 287</td>
<td>584 ± 91</td>
</tr>
<tr>
<td>Glutamic-oxaloacetic transaminase</td>
<td>4</td>
<td>24,660 ± 1,140</td>
<td>23,640 ± 1,230</td>
</tr>
<tr>
<td>Glucose 6-phosphate</td>
<td>4</td>
<td>2,590 ± 555</td>
<td>2,626 ± 476</td>
</tr>
</tbody>
</table>

Mean ± standard error.

**Table II**

**Effect of perfusion on enzyme leakage**

Part of the liver was removed before the perfusion and was homogenized. This homogenate was designated "unperfused liver." The rest of the liver was used as described in "Experimental Procedure."

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Unperfused liver</th>
<th>Perfused liver</th>
<th>Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamic-pyruvic transaminase</td>
<td>1614</td>
<td>1588</td>
<td>564</td>
</tr>
<tr>
<td>Serine dehydrogenase</td>
<td>54</td>
<td>54</td>
<td>0</td>
</tr>
<tr>
<td>Threonine dehydrogenase</td>
<td>47</td>
<td>46</td>
<td>0</td>
</tr>
<tr>
<td>Glucose 6-phosphate</td>
<td>2200</td>
<td>2160</td>
<td>2100</td>
</tr>
</tbody>
</table>

showed that the enzymes of the citrate cycle remained in the cells (13). In addition, about 35% of the glutamic-pyruvic transaminase, and 96% of the glutamic-oxaloacetic transaminase, remained in the cells.

The leakage of enzymes did not occur on perfusion of the liver, but on dispersion of the cells (Table II). It was also found that even when enzymatic methods were used for dispersion of the cells (such as the use of pancreatin, trypsin, or hyaluronidase (4, 5)) leakage of these enzymes was not prevented. Henley, Sorensen, and Pollard reported that the leakage of various enzymes could not be prevented by the addition of various substances during the dispersion of the cells (23). These substances included ATP, hemoglobin, and serum albumin. We also observed that enzyme leakage was not prevented by addition of ATP together with oxidizable substrates such as succinate or fumarate, or of dinitrophenol, to the medium used for perfusion and dispersion. In addition, when the cell suspension was prepared at 37°C, enzyme leakage was not prevented in the presence or absence of oxidizable substrates. These facts suggest that enzyme leakage from dispersed cells may not be energy-dependent. Therefore it seems possible that the leakage of enzymes from the cells, whatever the method used for dispersion, is due to a change in cell permeability.

As mentioned above, the enzymes which leaked out were all enzymes of the soluble fraction, and, as seen in Table I, approximately two-thirds of the glutamic-pyruvic transaminase activity of the original homogenate leaked out when the cells were dispersed. Since Katunuma et al. (24) reported that glutamic-pyruvic transaminase is present in both the soluble and mitochondrial fractions, and the enzymes in these two fractions have different properties, it was of interest to see which fraction of the glutamic-pyruvic transaminase leaked out. When the original homogenate was applied to a DEAE-cellulose column, glutamic-pyruvic transaminase activity was eluted in two peaks (Fig. 2). By preliminary chromatography of the supernatant and mitochondrial fractions of rat liver, the pattern of elution of the transaminases was elucidated (Fig. 3). Thus the first peak to be eluted was identified as that derived from the soluble fraction, while the activity of the second peak of the eluate represented mitochondrial activity. The activity ratio of the first and second peaks was approximately 3:1.

On the other hand, chromatography of the cell homogenate yielded two glutamic-pyruvic transaminase fractions in which the ratio of the activities of the soluble and mitochondrial fractions was 1:2.4 (Fig. 4). Since Henley et al. showed that there is not significant leakage of total protein from cells during their preparation (12), one can compare the activities before and after cell preparation on a dry weight basis. Thus one-fourth of the activity of the original homogenate as shown in Table I (1900 μmoles) should be attributed to the activity of the mitochondrial fraction (i.e. 422 μmoles) while 2.4/3.4 or 70% of the activity of the dispersed cells (584 μmoles) represents mitochondrial activity (i.e. 412 μmoles). These calculated values for the mitochondrial activity of the two preparations agree well with the data obtained. This indicates that the majority of glutamic-pyruvic transaminase activity remaining in the dispersed cells is that of the mitochondrial enzyme, whereas almost all the soluble glutamic-pyruvic transaminase had leaked out.

To prove that the activity which leaked out of the cells was that from the soluble fraction, the supernatant fraction obtained after centrifugation of the filtrate of the cell preparation (see "Experimental Procedure") was chromatographed (Fig. 5). It

**Fig. 2. DEAE-cellulose column chromatography of glutamic-pyruvic transaminase (GPT) in original homogenate.** The activity of the supernatant fraction applied was 1081 × 10³ μmoles of pyruvate formed per hour, and the protein content was 175 mg. The specific activity was 1780 mpmoles of pyruvate formed per hour per mg of homogenate, dry weight.
fraction is likely to be derived from cells ruptured during the content of the carcass and an increase in that of the liver (26, 27), been reported that glucocorticoids cause a decrease in the protein preparation of the cell suspension.

Low activity of mitochondrial enzymes found in the supernatant fraction of the homogenate contained an activity of 113.4 X 10^3 mpmoles of pyruvate formed per hour, and 136 mg of protein. and the increase in liver weight after glucocorticoid administration is due not to an increase in the cell number, but rather to the accumulation of nitrogenous substances in the cell (26). Moreover it was also suggested that the accumulation of nitrogenous substances is the result of changes in cell permeability rather than changes in the metabolism (28). It seemed probable, therefore, that glucocorticoids might also prevent the leakage of enzymes from liver cells in suspension.

Experiments were carried out to test this possibility. It was indeed found that when the rats had been treated with cortisone, a dispersion of their liver cells retained about twice as much glutamic-pyruvic transaminase as a dispersion of cells from untreated animals. Dexamethasone, a synthetic corticosteroid, caused a particularly marked retention of both glutamic-pyruvic transaminase and lactate dehydrogenase, even at less than one tenth of the dosage of cortisone (Table III). It should be added that there was no sex difference in the rats used for studies on the preventive effect of glucocorticoids on enzyme leakage.

The effect of dexamethasone on glutamic-pyruvic transaminase leakage was followed over a period of time, as shown in Fig. 6. Enzyme retention had already started to increase above the control value 1 hour after injection of dexamethasone, and it continued to increase to almost 100% of the control value by the 2 day. Thereafter the retention decreased up to the 5 day. The cause of this decrease is still unknown.

The addition of 1 mg of dexamethasone to 50 ml of the perfusion fluid did not cause a significant increase in the retention of enzymes.

The leakage of tryptophan pyrrolase and serine and threonine dehydrogenase could not be prevented by treatment with these hormones under the above conditions. It is interesting that the enzymes that are not prevented from leakage by dexamethasone are all readily inducible by this hormone (29-31).

Four days after dexamethasone treatment it was found that the total glutamic-pyruvic transaminase activity of the original homogenate had increased considerably. It was therefore interesting to see which fraction of the glutamic-pyruvic transaminase had increased in activity. Accordingly, the whole liver homogenate after dexamethasone treatment was chromatographed on a DEAE-cellulose column. It was found that there was more than 7 times more activity in the soluble fraction than in the mitochondrial fraction, as shown in Fig. 7, whereas for untreated liver the

was found that although there were two active fractions in the resulting eluate, the fraction representing the soluble part was far more active than that representing mitochondrial enzyme, the ratio of the two activities being 7.7:1, whereas this ratio in the original homogenate was 3:1, as shown in Fig. 2. Therefore it is evident that the loss of activity on cell dispersion is probably not due to enzyme inactivation, but to leakage of enzymes of the soluble fraction from the cell, and that enzymes of the mitochondrial fraction remain in the cells even after dispersion. The low activity of mitochondrial enzymes found in the supernatant fraction is likely to be derived from cells ruptured during the preparation of the cell suspension.

Effect of Glucocorticoids on Enzyme Leakage from Cells—It has been reported that glucocorticoids cause a decrease in the protein content of the carcass and an increase in that of the liver (26, 27),

and for the untreated liver the

**Figure 3.** DEAE-cellulose column chromatography of glutamic-pyruvic transaminase (GPT) in supernatant and mitochondrial fractions of rat liver. Normal rat liver was homogenized and fractionated to subcellular fractions, according to the method of Hogeboom (25). The protein of the supernatant fluid was precipitated by addition of solid ammonium sulfate to saturation, and the precipitate thus formed was dissolved in a small volume of 0.01 M potassium phosphate buffer, pH 7.0. The mitochondrial fraction was sonically disrupted. Both fractions were dialyzed before application to the column. The conditions of sonic treatment and dialysis are described under "Experimental Procedure." These fractions were applied to columns separately but are presented in the same figure for comparison. O—O, activity of the supernatant fraction (total activity, 1070 X 10^3 mpmoles; protein, 191 mg); and Q—O, mitochondrial activity (total activity, 336 X 10^3 mpmoles; protein, 71.2 mg).

**Figure 4.** DEAE-cellulose column chromatography of glutamic-pyruvic transaminase (GPT) of the cell homogenate. The supernatant fraction of the homogenate contained an activity of 113.4 X 10^3 mpmoles of pyruvate formed per hour, and 136 mg of protein.

**Figure 5.** DEAE-cellulose column chromatography of glutamic-pyruvic transaminase (GPT) which leaked out from the cells. The supernatant fraction of the crude filtrate contained an activity of 730 X 10^3 mpmoles of pyruvate formed per hour, and 188 mg of protein.
TABLE III

Effect of glucocorticoids on enzyme leakage from rat liver cells

<table>
<thead>
<tr>
<th>Hormone</th>
<th>No. of animals</th>
<th>Enzyme</th>
<th>Enzyme activity (mmoles/hr/mg dry homogenate)</th>
<th>Retention %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>Glutamic-pyruvic transaminase</td>
<td>1650 ± 287</td>
<td>35 ± 4.6</td>
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<tr>
<td>Cortisone</td>
<td>11</td>
<td>Glutamic-pyruvic transaminase</td>
<td>2412 ± 312</td>
<td>1470 ± 186</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>5</td>
<td>Glutamic-pyruvic transaminase</td>
<td>3312 ± 303</td>
<td>2263 ± 147</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lactic dehydrogenase</td>
<td>3150 ± 240</td>
<td>1200 ± 60</td>
</tr>
</tbody>
</table>

The values are cited from Table I. Mean ± standard error.

Cortisone (10 mg) was injected daily into each rat for 4 days, and the rats were killed on the 5th day.

Dexamethasone (1 mg) was injected into each rat daily for 4 days, and the rats were killed on the 5th day.

Comparison of Effects of Various Corticosteroids on Leakage of Glutamic-Pyruvic Transaminase—Thomson and Mikuta (31) reported that the induction rate of tryptophan pyrrolase by cortisol and cortisol was proportional to the dose of hormone administered. It would also be mentioned that these glucocorticoids have an anti-inflammatory action (33). Therefore it was interesting to see whether the effects of the corticosteroids on tryptophan pyrrolase induction, anti-inflammatory activity, and prevention of enzyme leakage were interrelated. Table IV shows that when a small dose of the corticosteroids was administered, cortisol and cortisol, which have the same degree of anti-inflammatory potency, showed no effect on either enzyme leakage or tryptophan pyrrolase induction, whereas the same dose of dexamethasone increased tryptophan pyrrolase activity in the soluble but not in the mitochondrial fraction. A similar phenomenon was reported on induction of phosphoenolpyruvic carboxykinase (32).

Comparison of Effects of Various Corticosteroids on Glutamic-Pyruvic Transaminase Activity—Portions (0.5 mg) of the hormone indicated were administered to the rats by injection and the animals were killed after 3 hours.

Fig. 7. DEAE-cellulose column chromatography of glutamic-pyruvic transaminase (GPT) in the original homogenate after treatment of animals with dexamethasone. Dexamethasone (1 mg) was injected into rats daily for 4 days, and the animals were killed on the 5th day. The original homogenate contained an activity of 1860 × 10³ mmoles of pyruvate formed per hour, and 125 mg of protein. The specific activity was 3720 mmoles of pyruvate formed per hour per mg of homogenate, dry weight.

TABLE IV

Comparison of effect of various glucocorticoids on glutamic-pyruvic transaminase retention and tryptophan pyrrolase induction

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Glutamic-pyruvic transaminase activity (mmoles/hr/mg dry homogenate)</th>
<th>Retention %</th>
<th>Tryptophan pyrrolase activity of original homogenate (mmoles/hr/mg dry homogenate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dexamethasone</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 6. Time course of the effect of dexamethasone on glutamic-pyruvic transaminase (GPT) retention in the cells. Dexamethasone (1 mg) was injected into each rat per day. Figures in parentheses represent the numbers of rats used.
Enzyme leakage, which has a much higher anti-inflammatory potency than cortisone and cortisol, prevents enzyme leakage from intact liver cells in suspension. Henley et al. (11, 12) showed that enzymes such as glutamic-pyruvic and glutamic-oxaloacetic transaminases, and lactic dehydrogenase, which Henley et al. had reported, the cellular activities of tryptophan pyrrolase and serine and threonine dehydrases were completely excluded. This prevention of enzyme leakage from cells by glucocorticoids may be responsible for the increase in liver weight, without a comparable increase in cell number, in rat liver after treatment with these hormones (26, 27).

With regard to the effects of corticosteroids on permeability, there are references in the literature showing that glucocorticoids, such as cortisone and cortisol, prevent enzyme leakage from thymocytes (43) and lysosomes (44). Sex hormones and deoxycorticosterone acetate have the reverse effect.

This prevention of enzyme leakage from cells by glucocorticoids may be responsible for the increase in liver weight, without a comparable increase in cell number, in rat liver after treatment with these hormones (26, 27). There is evidence suggesting that the induced increase of enzyme activity is due to an increase de novo in the number of enzyme molecules as a result of stimulation of enzyme synthesis (45-47). However, the possibility that enzyme increase may be partly due to accumulation of enzymes within the cell, as a result of a change of cell permeability, is not completely excluded.

The mechanism of the preventive action of glucocorticoids on enzyme leakage is not yet clear, although it is very likely that the hormone may act on the cell membrane, causing a change in permeability. However, it is unlikely that this change involves protein synthesis, since puromycin does not abolish the action of dexamethasone on enzyme leakage.

Since the enzymes which leaked out completely are localized in the soluble fraction of the cell, and since glucose 6-phosphatase and enzymes of the citrate cycle, which stayed in the suspended cells (13), are particle-bound or mitochondrial enzymes, it seems reasonable to assume that the enzymes in the hyaloplasm may have a tendency to leak through the cell membrane during the dispersion process. Recently Berry (41) found that glucose 6-phosphatase leaked out to a considerable degree from dispersed cells of mouse liver. This observation is in contrast to the results obtained in the present work and those of Henley et al. (23). Since Berry and Simpson (42) reported subsequently that their preparations of cells showed considerable damage of the cell membranes, it is likely that the discrepancy may be due to the degree of cell damage.

It appeared probable that the activity of glutamic-pyruvic transaminase remaining in the cells after dispersion might be that of enzyme localized in mitochondria, since Katunuma et al. (24) showed that the glutamic-pyruvic transaminase activity of rat liver is distributed in both the mitochondria and the soluble part of the cell. Our study indeed showed that the activity of glutamic-pyruvic transaminase which leaked out from the cells was that from the soluble fraction, and the activity remaining in the cells was due to mitochondrial enzyme.

Although no extensive morphological investigations were carried out in the present work, there are a number of reports showing that cell suspensions prepared in the way we have described do not show any microscopic evidence of damage or change (6, 14, 21), and that the leakage of enzymes is not due to a breakage of the cells, but to changed permeability of the cell membrane (11, 12). Moreover, using ascites tumor cells, Holmberg (40) showed that leakage of enzymes actually occurs from living cells. Wu (38) reported that there is a difference in the degree of leakage of glycolytic enzymes. Leakage of enzymes from cells occurs whatever method is used for their preparation.

With regard to the effects of corticosteroids on permeability, there are references in the literature showing that glucocorticoids, such as cortisone and cortisol, prevent enzyme leakage from thymocytes (43) and lysosomes (44). Sex hormones and deoxycorticosterone acetate have the reverse effect.

The prevention of enzyme leakage from cells by glucocorticoids may be responsible for the increase in liver weight, without a comparable increase in cell number, in rat liver after treatment with these hormones (26, 27). There is evidence suggesting that the induced increase of enzyme activity is due to an increase de novo in the number of enzyme molecules as a result of stimulation of enzyme synthesis (45-47). However, the possibility that enzyme increase may be partly due to accumulation of enzymes within the cell, as a result of a change of cell permeability, is not completely excluded.

The mechanism of the preventive action of glucocorticoids on enzyme leakage is not yet clear, although it is very likely that the hormone may act on the cell membrane, causing a change in permeability. However, it is unlikely that this change involves protein synthesis, since puromycin does not abolish the action of dexamethasone on enzyme leakage.

The present work indicates some relationship between enzyme leakage and inflammation. Recent clinical observations show that many enzymes appear in the serum of patients with hepatitis and these are very similar to those which leak out from cells in suspension (48). Glucocorticoids also prevent the appearance of these serum enzymes (49, 50). There is ample evidence suggest-

### Table V

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glutamic-pyruvic transaminase activity</th>
<th>Retention of glutamic-pyruvic transaminase</th>
<th>Tryptophan pyrrolase activity of original homogenate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Original homogenate</td>
<td>Cell homogenate</td>
<td>%</td>
</tr>
<tr>
<td>None</td>
<td>1690 ± 287</td>
<td>584 ± 91</td>
<td>96 ± 4.6</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>1770 ± 400</td>
<td>552 ± 324</td>
<td>85 ± 9.2</td>
</tr>
<tr>
<td>Puromycin</td>
<td>2222 ± 228</td>
<td>912 ± 66</td>
<td>41 ± 1.0</td>
</tr>
<tr>
<td>Dexamethasone + puromycin</td>
<td>3106 ± 340</td>
<td>1684 ± 318</td>
<td>78 ± 9.8</td>
</tr>
</tbody>
</table>

* The results of the control experiment are cited from Table I.
* Mean ± standard error.

DISCUSSION

Although there have been many reports on enzyme leakage from ascites tumor cells (37-40), few papers are available on enzyme leakage from intact liver cells in suspension. Henley et al. (11, 12) showed that enzymes such as glutamic-pyruvic and glutamic-oxaloacetic transaminases, and lactic dehydrogenase, leaked out from suspensions of rat liver.

In the present work it was shown that, besides the enzymes which Henley et al. had reported, the cellular activities of tryptophan pyrrolase and serine and threonine dehydrases were completely lost by leakage. Since these enzymes remained after perfusion of the liver, but had leaked out by the first centrifugation after filtration, it is very likely that leakage occurred at the time of dispersion. This accords well with results reported by Henley et al. (11) and Berry (41).
ing that enzyme increase in the blood can occur in the absence of tissue necrosis when there is an alteration in permeability (51).

**SUMMARY**

Rat liver cells were dispersed in suspension, and the activities of various enzymes were compared before and after dispersion. It was found that more than 90% of the glucose-6-phosphatase and of the glutamic-oxaloacetic transaminase remained in the cell, whereas tryptophan pyrrolase, and serine and threonine dehydrases, were completely lost when the cells were dispersed. Only 35% of the glutamic-pyruvic transaminase remained in the dispersed cells, and the rest of the activity leaked out. The activity which leaked out represented that from the soluble fraction, and the activity which remained in the cells was mitochondrial activity, as shown by chromatography on diethylaminoethyl cellulose columns.

Glucocorticoids such as cortisone, cortisol, and dexamethasone (16α-methyl-9α-fluoro-1-dehydrocortisol) had a preventive action on leakage of glutamic-pyruvic transaminase and lactate dehydrogenase. Their actions on enzyme leakage were parallel with their anti-inflammatory potencies. Thus dexamethasone showed the most effect and deoxycorticosterone acetate had no effect on enzyme leakage.

Glucocorticoids such as dexamethasone increased glutamic-pyruvic transaminase activity in the soluble, but not in the mitochondrial fraction of rat liver.

Puromycin did not inhibit the preventive action of dexamethasone on enzyme leakage.

**REFERENCES**
