Oscillating sperm that were not rotating did not cause peaks in the horizontal sweep of the oscillograph (Fig. 1*D*).

Although the method in which the multiplier photocell was used gave the clearest recording of the type of sperm activity that was occurring, it did fail to give a measure of the total number of sperm present. This might be overcome by means of a mechanically operated stage which would permit counting the sperm in a known area of the slide by scanning with the multiplier phototube.

While none of the methods tried thus far have given results which approach the ideal for evaluating sperm motility, all the methods show considerable promise and are being investigated further. Relatively complex equipment was used for these tests as a matter of expediency. Simplified equipment to accomplish the same purposes is being assembled.

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Analysis of Small Amounts of Fatty Acids

A recent note by C. M. Coleman and G. Middlebrook (*1*) describes a new method for estimating small amounts of fatty acids. The authors claim that their method is the best described to date. The purpose of the present note is to draw the attention of the authors and of others working with fatty acids to a widely applicable general method published last year (*2*). In this method, as in that of Coleman and Middlebrook, ionic dyes of opposite charge to large organic ions are used in a two-phase system, consisting of water and an organic liquid. Unlike the method of Coleman and Middlebrook, in which use is made of the interfacial enrichment of the dye, this method makes use of the preferential partition of the stoichiometric simple salts that dyes form with large organic ions (*3*) into the organic phase. This makes the method an equilibrium method, in contrast to that of Coleman and Middlebrook. The method is, moreover, considerably more sensitive and accurate than that of Coleman and Middlebrook. For lauric acid, for example (a system for which their method did not give any result), estimation as the sodium salt of about 20 mμmole to approximately 1-percent accuracy can be carried out through the use of pinacyanol under proper conditions (*2*).

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We wish to thank Mukerjee for having called his paper of 1956 to our attention. At the time of submission of our paper for publication in *Science*, reference to Mukerjee's work had not yet been indexed under a subject heading in *Chemical Abstracts*, and, consequently, we missed the original publication.

We have tried Mukerjee's method and agree with him that it is an excellent method not only for measuring small amounts of long-chain fatty acids but for measuring many other ionic surfactants. We would like to point out, however, that our method, in our experience, is more specific for the analysis of long-chain fatty acids in mammalian blood and tissue lipids. For example, phospholipids do not interfere with the determination of long-chain fatty acids in our method.

Our ignorance of Mukerjee's work very aptly points up the need for more prompt and efficient centralized transfer of information between investigators.

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An Enzymatic Basis for the Gluconeogenic Action of Hydrocortisone

The pronounced effects of cortisone and related steroids on carbohydrate metabolism appear to be due to their activity on hepatic gluconeogenesis rather than on the peripheral utilization of glucose. However, the biochemical mechanisms mediating the gluconeogenic action of these compounds are not known. Recently, Gavosto *et al.* (*1*) suggested that cortisone increases gluconeogenesis and imposes a negative nitrogen balance by enhancing transamination processes. They observed that near-toxic doses of cortisone (120 mg/kg), when administered to rats for 3 days, increased the activity of glutamic-oxalacetic transaminase (GOT) by 67 percent and that of glutamic-pyruvic transaminase (GPT) by 81 percent in liver. Independently, in the course of studies on the effect of hydrocortisone on enzymes which require pryidoxal phosphate as a cofactor, we have noted as much as 500 percent increase in the GPT activity in livers of rats treated with hydrocortisone, whereas, under the same conditions, the values for GOT were only slightly higher than those of the untreated control animals.

Male albino rats (Holtzman) weighing from 125 to 150 g were used. The methods of Lowry and co-workers for GOT (*2*), lactic acid dehydrogenase (*3*), and protein (*4*) were used. The method used for GPT (*5*) is based on the same principle as that of Wroblewski and LaDue (*6*), except that instead of measuring spectrophotometrically the disappearance of reduced diphosphopyridine nucleotide (DPNH), the DPN formed is determined fluorometrically (*3*). Tissues were kept at 0°C until they were homogenized and assayed. These enzymes were found to be stable during storage at −20°C in the 1 to 20 homogenates of brain and liver.

The marked rise in GPT activity, in contrast to the activity of GOT, in the liver of rats receiving hydrocortisone, is shown in Table 1. Lactic acid dehydrogenase (LDH), an enzyme which does not require pyridoxal phosphate, was not affected by treatment with hydrocortisone. Similar analyses of whole brain from the same animals revealed less than 20 percent increase in GPT activity and no significant changes in GOT and lactic acid dehydrogenase values associated with treatment. When pyridoxal phosphate was added to the complete system for each transaminase, no stimulatory effect could be detected. It was also noted that the addition of hydrocortisone in vitro to the GPT homogenate system did not enhance the activity of this transaminase.

In an earlier experiment with pyridoxine-depleted rats, GPT values ranging from 500 to 700 percent above normal were observed after 4 weeks of treatment with 5 mg of hydrocortisone per rat per day. In the studies summarized in Table 1, the GPT levels were doubled after 2 days of treatment, and maximum GPT activity in liver occurred after the seventh day of treatment with 2.5 or 5 mg of hydrocortisone per day. Although some variation in the control values for GPT was noted on different days of the experiment, the standard deviation of each group remained small. On the 28th day, 14 days after the administration of hydrocortisone was discontinued, the GPT activity was still considerably higher than normal. Whether this observation can be attributed to the normal turnover rate of this enzyme in liver, or to the prolonged retention of hydrocortisone or an active metabolite, cannot be answered at this time.

The administration of large amounts of pyridoxine to animals receiving hydrocortisone did not alter the magnitude of the GPT response. Furthermore, depletion of rats of pyridoxine for 8 weeks did not impair the increase in GPT following daily injections of hydrocortisone (5 mg) for 1 week. Thus, neither depletion of pyridoxine nor administration of this vitamin appears to affect the changes in hepatic GPT levels produced by hydrocortisone.

Table 1. Effect of two levels of hydrocortisone on glutamic-pyruvic transaminase (GPT), glutamic-oxalacetic transaminase (GOT) and lactic acid dehydrogenase (LDH) activities of rat liver. There were 20 animals in each group; five animals sacrificed on days 2, 7, 14, and 28. The animals were maintained on Purina mouse diet. The weight of rats in the untreated group was controlled during days 1 to 14 by restricting diet; thereafter, the rats were allowed to eat *ad libitum*. Hydrocortisone was injected subcutaneously (2.5 and 5.0 mg per rat per day). The administration of hydrocortisone was discontinued on day 15. The animals were killed by severance of the carotid artery and were exsanguinated before the tissues were removed.

Hydrocortisone treatment (mg)	Enzyme activity (mmole of substrate utilized/g of protein, per hour at 38°C)		
	GPT	GOT	LDH
	Second day		
0	12.1 ± 3.2*	107 ± 10	281 ± 12
2.5	18.9 ± 2.0	112 ± 9.2	271 ± 12
5.0	25.4 ± 3.4	103 ± 5.2	259 ± 9.0
	Seventh day		
0	8.7 ± 2.9	128 ± 3.6	290 ± 19
2.5	44.7 ± 4.1	140 ± 8.9	277 ± 33
5.0	53.0 ± 5.1	157 ± 3.9	268 ± 19
	Fourteenth day		
0	16.1 ± 1.8	122 ± 13	266 ± 6.0
2.5	66.4 ± 4.4	152 ± 5.8	247 ± 25
5.0	71.0 ± 2.3	142 ± 14	242 ± 24
	Twenty-eighth day		
0	17.5 ± 2.0	143 ± 6.8	299 ± 16
2.5	30.3 ± 4.3	157 ± 2.8	246 ± 16
5.0	52.5 ± 2.0	172 ± 7.7	226 ± 14

* Standard deviation = $(\Sigma d^2/n)^{\frac{1}{2}}$.

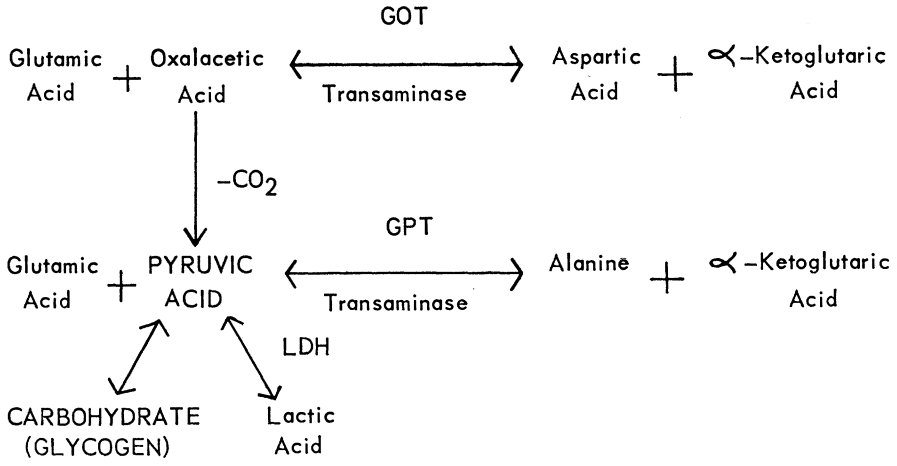

Fig. 1. Transaminase reactions and pathway to carbohydrate synthesis.

It was of interest to determine whether other corticosteroids increased hepatic GPT activity to the extent observed with hydrocortisone. The daily subcutaneous injection of rats with Prednisone (2.5 mg) or cortisone acetate (5 mg) for 1 week resulted in more than a five-fold increase in liver GPT activity in each case. In a comparable experiment, deoxycorticosterone was administered (3 mg daily); the hepatic GPT values were not increased, and they appeared to be somewhat lower than those of the untreated control animals. The relationship between the stimulation of hepatic GPT activity and the gluconeogenic potency of related steroids is under study to evaluate the hepatic GPT response to corticosteroids as a method of assay.

Negative nitrogen balance is also an important aspect of treatment with glucocorticosteroids. These studies do not indicate whether the effect of hydrocortisone is limited to alterations in GPT activity or whether transamination processes other than GOT are also affected. It is apparent, however, that increased transaminase activity can be related directly to amino acid imbalances which initiate protein catabolism and negative nitrogen balance.

Of the many amino acids which have been studied for gluconeogenic activity, alanine, aspartic acid, and glutamic acid are unique with regard to their high gluconeogenic potency (*7*). The relationship between the substrates in the transaminase reactions studied herein and the pathway to carbohydrate synthesis is shown in Fig. 1. Pyruvic acid appears to be the common intermediate in the conversion of these amino acids to glycogen. Both pyruvic acid and lactic acid are readily converted to carbohydrate. Moreover, both of these metabolites occur in elevated concentrations in the blood of patients with Cushing's syndrome and in subjects receiving glucocorticosteroids (*8*). These facts, added to the observation that a substantial rise in hepatic GPT occurs in rats treated with hydrocortisone, in contrast to treatment with deoxycorticosterone, strongly suggests that the control of hepatic levels of GPT by glucocorticosteroids is importantly related to the mechanism whereby these compounds exert their gluconeogenic activity (*9*).

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Corrosion of Pure Aluminum and Tin in Salt Spray

Despite the widespread and continued use of the salt spray test as a specification for plated metal parts (*1*) and as an accelerated corrosion test (*2*), very little is known about the actual mechanism of corrosion under these conditions. In