in concentration of agent or of exposure period percentages of ectodermized forms decrease, those of differentially inhibited forms increase. This is what would be expected from an inhibitory agent. The fact that cleavage and development are retarded further indicates the inhibitory action of NaCNS. Ectodermized forms result only when the treatment is sufficiently light to permit recovery of some individuals yet strong enough to affect the primary pattern. The exposure of unfertilized eggs to NaCNS in Ca-free sea water appears to result in a differential inhibition of the normal axiate pattern. With recovery on return to normal sea water and fertilization the basal levels of the egg seem to be freed to some extent from apical influence and develop in the same, or almost the same, direction as the originally higher levels. In other words the whole egg now develops as the animal portion with an increased scale of organization and a diminution or absence of entoderm and mesenchyme. When the early developmental stages (*i.e.*, first 12 hours after fertilization) are spent in solutions of NaCNS the resulting entodermization seems to follow from the depression of the more basal levels of prospective ectoderm to the physiological level of prospective entoderm. Such entodermized ectoderm may be considerably increased in size as a result of recovery on return to normal sea water. The increase in what appears to be mesenchyme results largely or wholly from dissociation and passage into the blastocoel of cells from the original entoderm. Child[4] has given a similar interpretation for entodermized larvae resulting from treatment with lithium.

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Variations in Arginase Activity in Livers of White Rats, Caused by Fasting.

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Baldwin[1] has indicated that the liver arginase of some animal species is dependent upon the nutritional condition. Takehara[2] determined arginase in the livers and the kidneys of 4 male dogs after fasts of 7 to 23 days. He concluded that "the value for arginase in liver and kidney is markedly increased by fasting." The writers

1 Baldwin, E., *Biol. Rev.*, 1936, **11**, 247.

2 Takehara, H. J., *Biochem.* (Japan), 1938, **28**, 309.

have statistically analyzed the results of Takehara and find that the differences between the means are not significant.

The object of this investigation was to study the variations, caused by fasting, in the quantities of arginase present in the livers of white rats.

Procedure. Methods of care of the animals, the collection of samples, and the estimation of the arginase activity have been described elsewhere.[3]

Five groups of rats were studied and all data concerning them are presented in Table I. The animals, with the exception of those in the large group of males fasted for 5 days, were between 92 and 104 days old when sacrificed. The age range of the large group was 102 to 136 days. This wider age spread was necessary in order to obtain the desired range in body weights.

The different periods of fast were selected because of the relationship that has been shown between the quantity of liver arginase and the quantity of protein catabolized.[4] The 2-day fast period was selected because it has been shown by Addis, Poo and Lew[5] that a 2-day fast markedly reduces the protein content of the livers of white rats. The 8-day fast for one group of males and one of females was selected because it represents approximately the maximum survival time of rats under the conditions used in these experiments. A fast of 5 days for a group of males was selected since preliminary experiments had shown that it represents a stage of fasting previous to the time of onset of the "premortal rise" in nitrogen excretion. The number of animals in this group was large and statistical methods were used as an aid in interpretation of the data. Females were selected for the fifth group because it has been shown[3, 4] that livers of female rats at ages of about 100 days contain less arginase than do those of male rats of similar age. The 4-day fast was selected for this group since we were interested in the adjustment in the concentration of liver arginase by females during a time when the store of protein in the liver has been largely depleted, as indicated by the work of Addis, Poo and Lew,[5] and while there yet remains a considerable quantity of body fat.

Discussion. Adaptations in the quantity of liver arginase to the quantity of catabolized protein nitrogen produced by changes in protein consumption are reflected in a change in the size of the liver, which appears first, and in the concentration of the enzyme in the liver, which follows.[4] Evidence of a similar twofold adjustment

3 Lightbody, H. D., *J. Biol. Chem.*, 1938, **124**, 169.

4 Lightbody, H. D., and Kleinman, A., *J. Biol. Chem.*, 1939, **129**, 71.

5 Addis, T., Poo, J., and Lew, W., *J. Biol. Chem.*, 1936, **115**, 117.

may be seen in the results of the experiments reported in this paper. The concentrations of liver arginase were somewhat increased in male rats fasted for 2 days, but the size of the organ decreased about 28%, resulting in a marked decrease in the total quantity of the enzyme per unit of body weight. Females fasted for 4 days reacted in a similar manner, but failed to show an increase in the concentration, the total arginase per unit of body weight being reduced even more than in male rats which were fasted for 2 days. Adjustments by the sexes to prolonged fasts of 8 days were likewise made by somewhat different means. For example, the quantity of liver tissue per 100 g of body weight of male rats was found to have further decreased and the amount of enzyme per unit of weight of liver markedly increased. The sizes of the livers of the female rats were maintained from the fourth to the eighth day of fasting and the adjustment indicated solely by increase in the enzyme concentration. The quantity of liver arginase per unit of body weight after a fast of 8 days was found to be equal to, or exceed that in livers of rats fed a diet containing 25% milk proteins, though in neither sex was the concentration as great as that found after feeding a 75% protein mixture.[4]

The results obtained after fasting male rats for 5 days (Fig. 1) indicate that a definite relationship exists between body weight (used here as an index of reserve nonprotein energy materials) and the amount of liver arginase per unit of body weight. The smaller animals were found to contain quantities equal to or greater than the quantities for those animals of comparable ages permitted food near optimum in protein content.[4] In some cases the amount of enzyme was found to approach that found after fasts of 8 days. In contrast, heavy animals have smaller quantities of the enzyme per unit of body

TABLE I.
Influence of Fasting on the Arginase Content of Liver.
(With the exceptions noted, the values are group means.)

Sex	No. of animals	Duration of fast, days	Body wt loss, %	Livers			
				Solids, %	Dry, per 100 g rat, g	*Arginase units	
						Per mg dry liver	Per 100 g rat $\times 10^{-3}$
M	15	2	11.93	33.0±0.3	0.83±0.01	253.1± 6.3	211.1±6.7
M	18	8	32.05	33.0±0.3	0.75±0.02	337.8± 9.8	252.3±6.4
F	15	8	30.58	32.1±0.3	0.83±0.02	283.7±12.5	234.9±9.8
M	26	5	16.11-22.50†	30.2-35.9†	0.65-1.10†	166.3-317.2†	120.8-249.5†
F	14	4	18.89	32.9±0.2	0.83±0.02	197.7± 8.3	164.7±9.5

*The arginase unit is that quantity of the enzyme which will liberate urea equivalent to one micromole (M $\times 10^{-6}$) of carbon dioxide under the experimental conditions used.

†Range in values.

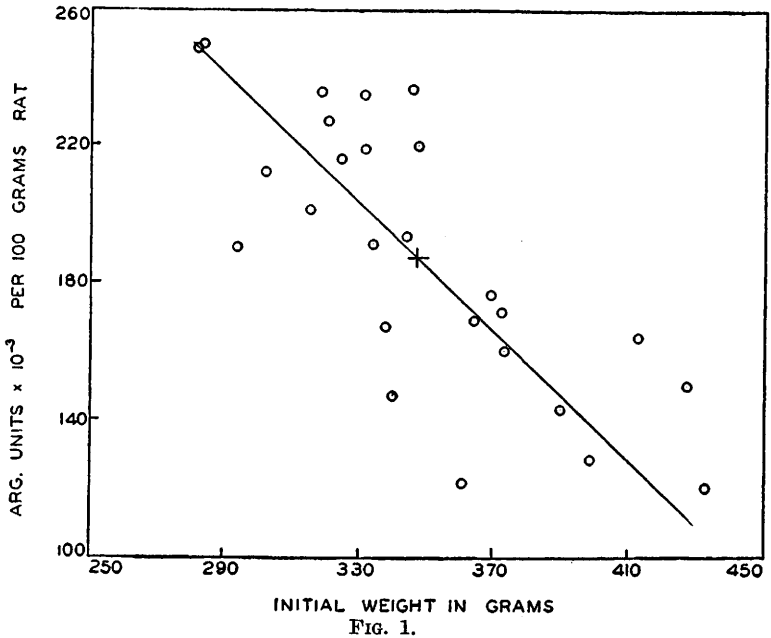

FIG. 1.

The regression line, as calculated by the method of least squares, from the results obtained by fasting male rats for 5 days.

weight, after a fast of 5 days, than have any yet examined during these studies.

If the quantity of liver arginase per unit of body weight is used as an index of the demands placed upon the enzyme system concerned with protein catabolism, the response of male rats to prolonged fast may be divided into 3 periods. The first of these is characterized by decrease in total liver arginase per unit of body weight reflected by a decrease in the quantity of liver tissue while maintaining the concentration. The second period, one of variable duration, is indicated by further decreases in the size of the liver and of the quantity of enzyme per unit weight of liver tissue. Finally there ensues a time after prolonged fast when the concentration of the enzyme in the liver is markedly increased, and the quantity of the enzyme present per unit of body weight may become equal to or greater than that found in animals fed food near optimum in protein content.[4] The adjustments in female rats when fasted 8 days seem to have been effected in manners similar to those in males.

Hutchinson and Morris[6] suggest that "either the caloric requirements immediately following starvation may be higher than normal requirements, resulting in the deamination of amino acids for energy

[6] Hutchinson, J. C. D., and Morris, S., *Biochem. J.*, 1936, **30**, 1695.

purposes, or the ability of the animal to retain absorbed N may be somewhat impaired following a prolonged period of undernutrition." In view of our findings we feel that the second possibility is the more likely explanation. The ability to utilize amino acids (as indicated by the ability to dispose of the waste nitrogen as urea) upon refeeding would appear to be somewhat dependent on the duration of the fast. If the fast were terminated and protein feeding resumed during a period when chiefly nonprotein materials were being used as the energy sources and at a time when the metabolic enzyme systems were adjusted to the utilization of carbohydrates or fats, the expected result would be poor utilization of the ingested protein, because of a deficiency of enzymes concerned with protein metabolism, and be reflected in nitrogen excretion characterized by low urea and high amino acid fractions. Efficient protein utilization at the new level of intake must be expected to await readjustment of the enzyme systems concerned in protein metabolism.

Conclusions. Rats respond to short fasts (2 days) without change in arginase concentration in the liver tissue, and adaptations to changes in metabolism are reflected in decreases in the size of the liver. If fasted until near the moribund condition there is increased arginase concentration in the livers of both sexes, with loss of sex differentiation. Adjustment of the quantities of this enzyme by male rats when fasted 8 days is reflected by change in the size of the liver and by increase in its concentration of the enzyme. Adjustments to long fasts by female rats is accompanied by increase in enzyme concentration and after 4 days of fast the quantities of liver tissue are not further decreased. As shown in Fig. 1 fasts of intermediate duration give variable results in the concentration of the enzyme in the livers of male rats. Such variations may be correlated with the type of body materials, carbohydrates and fats (non-nitrogenous) or proteins (nitrogenous), that serve as primary energy sources at the time of sacrifice. Expressed as quantity of enzyme per unit of body weight, arginase activity changes during the adjustment to the expected utilization of proteins as energy sources.