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Concerning the Site of Nitrogen Absorption in Rats Fed Autoclaved or Raw Soybean Oil Meal[1]

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Carroll, Hensley, and Graham (*1*) have concluded that much of the nitrogen absorption in rats fed raw soybean oil meal must take place in the cecum. This conclusion was reached from data showing that the apparent digestibility of raw soybean nitrogen in the terminal 20% of the small intestine was 32.65%, whereas in the feces the value was 76.96%. Values reported for apparent digestibility of heated soybean nitrogen were 78.66% and 81.78%, respectively. This observation presented a notable advance in explaining the lower nutritive value of raw soybeans compared with autoclaved soybeans. It therefore seemed advisable to repeat this work in order to determine the validity of the observations made.

The Cr_2O_3 index procedure was employed in a manner similar to that of Carroll *et al.* (*1*), with the following pertinent notations. The autoclaved and raw soybean rations contained 2.1% total nitrogen and were compounded as in previous studies (*2*). Rats of the Sprague-Dawley strain were fed the respective ration for a period of 4 days before being sacrificed.

TABLE 1

Apparent Digestibility of Soybean Nitrogen in the Terminal 20% of the Small Intestine

Series	No. of rats	Av wt (g)	Apparent digestibility ± SE (range) Soybean oil meal		t value
			Autoclaved (%)	Raw (%)	
1	20	126	73.35 ± 1.92 (52.7–84.8)		1.968*
	20	120		66.90 ± 2.66 (43.3–82.8)	
2	21	189	78.52 ± 0.93 (71.7–84.5)		1.644*
	18	186		75.17 ± 1.82 (59.1–86.5)	

* Not significantly different, t value according to Snedecor (*5*).

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The determination of Cr_2O_3 was carried out by the method of Schürch *et al.* (*3*), except that the dichromate color was read at 375 mμ, as suggested by Dansky and Hill (*4*).

The data accumulated in our experiments indicate that the apparent digestibility of raw soybean nitrogen was not significantly different from the apparent digestibility of autoclaved soybean nitrogen when determined by the Cr_2O_3 index method in the terminal 20% of the small intestine of the rat. The results are presented in Table 1.

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A Test Utilizing the *in Vitro* Clearing of Milk to Determine the Presence of Lipid Clearing Factor in Plasma

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Recent studies indicate that certain physically distinct lipoprotein particles suspended in the blood may be etiologically significant in atherosclerosis (*1, 2*). Following an earlier observation that heparin administered intravenously decreased the turbidity of plasma during alimentary lipemia (*3*), a number of workers have been investigating the possibility that the heparin clearing phenomenon may be of significance in lipid metabolism, particularly in relation to the etiology of atherosclerosis. The clearing effect has been found to occur *in vitro* when lipemic plasma is incubated with plasma withdrawn from donor animals soon after the intravenous administration of heparin (*4*). Subsequent work suggests that a soluble tissue substance, in the presence of heparin, catalyzes the conversion of a precursor present in plasma to a lipid clearing factor. The latter apparently effects a redistribution of plasma lipids in such a way that turbidity is decreased (*5*).

We have been testing for the presence of clearing factor precursor in the plasma of normal and abnormal subjects in connection with our work on postprandial serum turbidity in atherosclerotic patients (*6*). In the course of our study we have investigated the possibility of substituting for lipemic plasma a more easily procured and more readily standardized substance as testing material in the assay of lipid clearing factor. We have found that milk fulfills these requirements reasonably well.

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TABLE 1

CLEARING OF DILUTED MILK DURING INCUBATION WITH HEPARINIZED PLASMA

Min of incubation	Turbidity reading	
	Control mixture (nonheparinized plasma and diluted milk)	Test mixture (Heparinized plasma and diluted milk)
1	103	94
15	102	85
30	101	80
45	103	80
60	103	77
120	105	74

The procedure consists of a quantitative determination by turbidimetry of the decrease in turbidity obtained in milk when it is incubated with heparinized plasma. After a 12-hr fast a 15-ml control specimen of venous blood is obtained from the subject, and 100 mg of a solution of the sodium salt of heparin is given intravenously. No food is taken during the test period. Fifteen ml of venous blood is then collected at intervals that vary from 1 to 120 min after the administration of heparin. Each specimen is collected in an oxalated tube containing the evaporated residue of 1.5 ml 1.2% ammonium oxalate and 0.8% potassium oxalate. The specimens are centrifuged at 2000 rpm for 10 min, and the supernatant plasma is pipetted off. Three ml of each plasma specimen, including the control, are then thoroughly mixed with 1 ml of diluted fresh homogenized grade A milk previously diluted 1: 60 with physiologic saline and preheated to 37° C. The mixture of plasma and diluted milk is then incubated in a water bath at 37° C. Turbidity readings are made immediately after mixing and at various intervals during the next 24 hr in a photoelectric colorimeter, using microtubes. Distilled water is used as a blank, and a red filter (640–700 μ) is used to minimize serum color or slight degrees of hemolysis. We have expressed our results in terms of the direct scale readings[2] obtained in a Klett-Summerson photoelectric colorimeter.

Ten physically normal male psychiatric patients who varied in age from 18 to 30 years were tested. All showed the presence of lipid clearing factor by a variable decrease in turbidity of the diluted milk when it was incubated with heparinized plasma. Clearing activity could be demonstrated in plasma withdrawn as soon as 1 min after the intravenous injection of heparin. In general, maximum clearing activity was noted in plasma specimens withdrawn approximately 10 min after the administration of heparin. Following the incubation of the plasma and diluted milk mixture there was a gradual decrease in turbidity, with a maximum of clearing usually after 2 hr incubation. There was no significant change in the turbidity of the control specimens throughout the incubation. A typical assay of a plasma specimen collected 10 min after the administration of heparin is presented in Table 1.

A comparison of clearing factor activity in the 10 tested subjects revealed variable degrees of activity. In 2 subjects clearing activity was not noted until 1 and 2 hr, respectively, after the injection of heparin. In the remaining 8 individuals the presence of clearing factor was readily demonstrable in plasma specimens obtained 10 min after heparin injection. After 2 hr incubation turbidity decreased 5, 7, 13, 13, 14, 20, 21, and 28 scale units, respectively, in the plasma specimens of these subjects.

The results suggest that the procedure may be standardized as a useful clinical tool in evaluating the possible role of clearing factor precursor in the etiology of atherosclerosis. The study described here is being extended in an effort further to simplify the procedure and possibly to find other lipoprotein substances that may serve as more sensitive indicators of the presence of lipid clearing factor in plasma.

[2] Optical density values may be obtained by multiplying the direct scale readings by 0.002.

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Radioactive Iodine as an Indicator of the Uptake of Iodine by the Liver, Gastrocnemius, and Thyroid of *Rana pipiens*

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Iodine metabolism in body tissues has long been a problem, and until recently the only available methods for following the fate of iodine in the body were chemical determinations or biological assay of tissues. Today, the approach to the problem of iodine metabolism in body tissues can be simplified by the use of the radioactive isotope of iodine (I^{131}), which has a half-life of 8 days. With radioactive iodine tracer techniques it is possible to label the circulating iodine in the organs without altering the amount of iodine already present, since samples of radioactive iodine can be prepared practically free of iodine.

For the most part, such studies have dealt with the accumulation of iodine in the thyroid gland of warm-blooded animals. The present paper, on the contrary, is a report of experiments carried out on the frog to determine the concentration of iodine in hepatic and striated muscle tissue (gastrocnemius), as well as in thyroid tissue.

In these experiments male and female frogs (*Rana pipiens*) of 17–23 g body weight were kept in a