dexamethasone failed to increase NEFA levels and did not potentiate the lipemia produced by ACTH, and that corticotropin caused release of NEFA from adipose tissue *in vitro,* make it seem likely that the action of corticotropin in producing elevated plasma NEFA and total lipid content is an extra-adrenal one, not mediated by way of the adrenal cortex. This is consistent with the recent demonstration by Boright *et al.* of dissociation between the adrenal and extra-adrenal actions of corticotropin by periodate oxidation of the hormone(19).

Summary and conclusion. Repeated subcutaneous injections or intravenous infusion of corticotropin A_1 in rabbits caused prompt elevation of plasma NEFA levels, accumulation of fat in the liver and kidneys, and hyperlipemia. These responses appear to be evoked by extra-adrenal action of corticotropin A_1.

1. Rudman, D., Seidman, F., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 146.

2. Kellner, A., Hirsch, R. L., Freeman, E. B., *J. Exp. Med.,* 1960, v112, 1.

3. Rudman, D., Reid, M. B., Seidman, F., Di Girolamo, M., Wertheim, A. R., Birn, S., *Endocrinology,* 1961, v68, 273.

4. Astwood, E. B., Barrett, R. J., Friesen, H., *Proc. Nat. Acad. Sci.,* 1961, v47, 1525.

5. Lelek, I., Nagy, D., Kadas, L., Virag, L., *Endokrinologie,* 1961, v41, 337.

6. Hollenberg, C. H., Raben, M. S., Astwood, E. B., *Endocrinology,* 1961, v68, 589.

7. Li, C. H., Geschwind, I. I., Dixon, J. S., Levy, A. L., Harris, J. I., *J. Biol. Chem.,* 1955, v213, 171.

8. Dole, V. P., Meinertz, H., *ibid.,* 1960, v235, 2595.

9. Fiske, C. H., SubbaRow, Y., *ibid.,* 1925, v66, 375.

10. Folch, J., Lees, M., Stanley, G. H. S., *ibid.,* 1957, v226, 497.

11. Burgers, A. C. J., *Endocrinology,* 1961, v68, 698.

12. Saffran, M., Schally, A. V., *Endocrinology,* 1955, v56, 523.

13. Peterson, R. E., *J. Biol. Chem.,* 1957, v225, 25.

14. Richards, J. B., Sayers, G., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 87.

15. Sayers, G., in *Hormones in Blood.* C. H. Gray, A. L. Bachrach, editors, Academic Press, N. Y., 1961, p185.

16. Feigelson, E. B., Pfaff, W. W., Karmen, A., Steinberg, D., *J. Clin. Invest.,* 1961, v40, 2171.

17. Landolt, R., Astwood, E. B., *Am. J. Physiol.,* 1961, v200, 841.

18. Kobernick, S. D., More, R. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 602.

19. Boright, H. A., Engel, F. L., Lebovitz, H. E., Kostyo, J. L., White, J. E., Jr., *Biochem J.,* 1962, v83, 95.

Received May 28, 1962. P.S.E.B.M., 1962, v111.

Bioassay of Essential Acids. Comparison of Effect of Triglycerides, Methyl and Ethyl Esters. (27761)

H. J. THOMASSON AND J. J. GOTTENBOS (Introduced by E. H. Ahrens, Jr.)
Unilever Research Laboratory, Vlaardingen, The Netherlands

In the bioassay of essential fatty acids (EFA) the fatty acids can be administered to animals (rats) in various forms, *e.g.,* as triglycerides, methyl or ethyl esters.

Preliminary observations have indicated that the response of the EFA-deficient rats is poorer if the fatty acids are in the form of methyl esters instead of triglycerides. In the present investigation the influence of the 3 above-mentioned forms of fatty acids was studied.

Several types of fatty acids were used, *e.g.,* EFA-inactive fatty acids (lauric, myristic and oleic acid) and the active linoleic acid, all in the above-mentioned forms.

Experimental. The EFA bioassay of Thomasson(1) was used. During 5 weeks newly weaned male Wistar rats were fed a fat-free diet *ad libitum*; drinking water was restricted to 14 ml/animal/day. At the end of the preliminary period the mean body weight of the animals was 115 g. The ani-

TABLE I. Mortality and Changes in Body Weight of EFA-Deficient Rats Dosed with Different Esters of Fatty Acids.

Fatty acids (dose/animal/day)	Ester forms: Triglycerides Mortality	Triglycerides Body wt, g	Methyl esters Mortality	Methyl esters Body wt, g	Ethyl esters Mortality	Ethyl esters Body wt, g
.2 ml lauric acid	0/7	+ 2	6/7	−42	4/7	−22
.2 ml myristic acid	0/7	−10	6/7	−24	6/7	−54
.2 ml oleic acid	1/7	− 6	5/7	−38	2/7	−40
Mean for inactive acids	1/21	− 4	17/21	−35	12/21	−36
20 mg linoleic acid made up to 0.2 ml with: lauric acid	0/7	+64	0/7	+43	0/7	+47
myristic acid	0/7	+59	0/7	+47	0/7	+42
oleic acid	0/7	+64	0/7	+36	0/7	+34
Mean for linoleic acid groups	0/21	+63	0/21	+42	0/21	+41

mals were divided into 18 groups of 7 rats each and continued on the same water intake. 0.2 ml of test material was administered to each rat 5 times a week for 4 weeks by pipette. The following preparations were tested: a) Lauric, myristic or oleic acid, each in the form of triglycerides, methyl or ethyl esters (9 groups); b) 20 mg linoleic acid* made up to 0.2 ml with lauric, myristic or oleic acid. These fatty acids, linoleic acid included, were also used in the form of triglycerides, methyl or ethyl esters (9 groups).

The acids used were isolated from natural sources and were 97-100% pure to analysis by gas-liquid chromatography.

Results. The table records changes in body weight and mortality. Deaths occurred almost exclusively in the groups of animals given methyl and ethyl esters of biologically inactive acids. Therefore, in these groups, body weights are not reliable.

The differences between the lauric, myristic and oleic acid groups are not significant. Body weights of groups given inactive acids decreased whereas those given linoleic acid increased. The mean response in 4 weeks after administering 20 mg linoleic acid was about 70 g.

Triglycerides caused significantly higher body weights than methyl and ethyl esters, and almost no mortality. This finding permits the conclusion that comparisons of activities of fatty acids in the EFA bioassay are correct only when all the acids are administered in comparable form (triglycerides or esters). In addition, the low death rate and constancy of the body weight level of the deficient animals make the use of triglycerides preferable to that of the other ester forms.

The cause of the poorer growth action of methyl and ethyl esters, with or without linoleic acid, is unknown. Various animals tested with methyl and ethyl esters of inactive acids showed redness and loss of hair around the mouth; this condition may explain in part their poor growth.

The observed differences in response between triglycerides and the methyl and ethyl esters are striking. Results reported in the literature dealing with much higher dosing levels have not shown this effect. Probably the explanation must be related to the deficiency status of the animals. Since the purity of the samples was very high, differences cannot be ascribed to contaminants in the test materials.

Summary. In bioassays of EFA, fatty acids can be administered in various forms, *e.g.*, as triglycerides, methyl or ethyl esters. Responses of the animals vary with the form of the acids. Therefore, comparable materials should be used. The triglyceride form has definite advantages.

* This dosage of linoleic acid is known to achieve a distinct growth-response in EFA-deficient rats.

1. Thomasson, H. J., *Int. Z. Vitaminforsch.*, 1953, v25, 62.

Received May 28, 1962. P.S.E.B.M., 1962, v111.