The current study was undertaken to confirm previous observations with reference to the length of time that Trichomonas vaginalis was capable of surviving in a droplet of vaginal discharge under natural conditions and also whether it was capable of multiplying in culture at the end of each test period. It is observed in Table I and Fig. 1 that trichomonads survived for 45 minutes in the discharges from all cases tested, that in 29 or 58% they survived for 3 hours and that survival was demonstrated in only 2 or 4% at the end of 6 hours.

Trussell(6) raises the question of the survival of trichomonads following desiccation. In the current study the drying droplets of discharge were sufficiently moist up to the 5-hour period to permit transferring by pipette to a microscope slide or culture tubes the amount of material required for testing. At the 6- and 7-hour intervals, however, the specimens had become so dry that it was necessary to add saline to the block before the material could be transferred. Since only one of the 50 specimens tested at 6 hours was positive by culture and none of the 7-hour specimens yielded either motile flagellates or positive cultures, it is concluded that trophozoites of *Trichomonas vaginalis* will not withstand complete desiccation.

The observations here presented, however, do indicate that trichomonads in droplets of vaginal discharge, deposited under natural conditions may survive sufficiently long to permit transfer to another individual where they may again be established. Although actual experimental transfer of the recovered trichomonads has not been made to volunteers during this study, previous experiments by one of the authors(10) and also by other investigators(6,18) have demonstrated that *Trichomonas vaginalis* established in culture can be implanted and produce vaginal trichomoniasis. In view of these findings careful personal hygiene is indicated.

Summary. The survival of *Trichomonas vaginalis* was determined by studying discharge from 50 untreated patients with vaginal trichomoniasis. Droplets of the discharge were placed on the enamel surface of wooden blocks and tested under natural conditions of drying at room temperature.

Tests at intervals ranging from 10 minutes to 7 hours were made by comparing the activity of the flagellates in direct microscopic examination with their ability to multiply in serial cultures. A close correlation was observed between the two procedures. The trichomonads survived in 100% of the tests at 45 minutes, in 96% at 1 hour, in 56% at 3 hours and in 4% at 6 hours. No survival was demonstrated at 7 hours.

These data, together with experimental and epidemiologic reports of other workers indicate that fomites contaminated with *Trichomonas vaginalis* may, under natural conditions, act as agents in the transmission of vaginal trichomoniasis.

18. Hesseltine, H. C., Wolters, S. L., and Campbell, A. J., *J. Inf. Dis.*, 1942, v71, 127.

Received June 19, 1950. P.S.E.B.M., 1950, v74.

Relation of Cortisone Pretreatment to Mobilization of Lipids to Liver by Pituitary Extracts.*† (18039)

LOUIS LEVIN AND RITA K. FARBER

From the Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York City

Since the initial report of Best and Campbell(1), it has been shown in many labora-

* The work reported in this paper was supported by a grant (RG-1474) from the Division of Research Grants and Fellowships of the U. S. Public Health Service.

† A preliminary report of this work was made before the Division of Biological Chemistry of the American Chemical Society, Philadelphia, April 11, 1950.

tories that crude pituitary extracts are able to cause a rapid increase in the amount of fat in the liver. It has further been demonstrated(2,3) that this accumulating fat originates from the fatty depots of the body, the overall phenomenon therefore consisting of a mobilization of depot fat to the liver. A number of attempts have been made to attribute this property of pituitary extract to various of the known pituitary hormones, but to date no definite evidence has been presented to prove that any one or another of the known pituitary hormones is responsible for the mobilization of fat to the liver.

Data presented elsewhere(4-7) have indicated that the property of mobilization of fat to the liver cannot be ascribed solely to the action of adrenocorticotrophic hormone (ACTH) acting via the usually accepted route, *e.g.*, by stimulation of the secretory activity of the adrenal cortex. For example, it has been shown(5-7) that none of the available adrenocortical steroids (including cortisone) is able to cause accumulation of fat in the liver. On the other hand, there is almost complete‡ agreement(5,6,7,10,11,12) that total adrenalectomy completely prevents fat mobilization by pituitary preparations which are active, in this respect, in intact animals. Thus there exists a considerable contradictory dilemma as to the function of the adrenal cortex in relation to mobilization of fat.

The data here presented provide a significant clue as to the mode of participation of the adrenal cortex. These data show that in order to produce mobilization of depot fat to the liver two hormonal factors are required: (a) an adequate supply of circulating 11-oxygenated steroid (whether endogenous or exogenous) before and/or during the time of fat mobilization; and (b) the actual triggering substance, an appropriate pituitary extract or hormone. The latter is apparently neither ACTH nor growth hormone, or alternatively and less likely, each of these hormones has the ability to cause the mobilization of fat in appropriately prepared animals. These data further conclusively prove that whatever the identity of the pituitary substance involved, its action in triggering mobilization of fat to the liver is not mediated via the adrenal cortex.

Experimental. Adult female mice of the Carworth Farms CFW strain were used as experimental animals and were killed by cervical fracture at the end of a 7 hour fasting period. Pituitary preparations, when administered, were given as a single subcutaneous injection at the start of the fasting period. When treatment with cortisone was used, it was begun 3½ days prior to sacrifice of the animal. The steroid was administered in 2 daily subcutaneous injections of 0.5 mg each (usually as a suspension of the steroid

1. Best, C. H., and Campbell, J., *J. Physiol.*, 1936, v86, 190.

2. Barrett, H. M., Best, C. H., and Ridout, J. H., *J. Physiol.*, 1938, v93, 367.

3. Stetten, D., and Salcedo, J., *J. Biol. Chem.*, 1944, v156, 27.

4. Levin, L., *Fed. Proc.*, 1949, v8, 218.

5. Levin, L., *J. Clin. Endoc.*, 1949, v9, 657.

6. Levin, L., Abst. of Proc., First Int. Cong. Biochem., Cambridge, England, 1949, p. 393.

7. Levin, L., Abst. of Papers, 117th Meeting, Am. Chem. Soc., Philadelphia, 1950, p. 13c.

‡ The only exceptions known to us are the papers of Szego and White(8) and of White(9), both dealing with the same data, in which it is reported that mobilization of fat to the liver was obtained after administration of incompletely purified growth hormone to adrenalectomized as well as to intact mice. We have not been able to confirm either finding with the 3 growth hormone preparations we have used to date. We believe that we can explain the discrepancy between results obtained in intact mice in the 2 laboratories. This will be done in a later publication. Preliminary results, now being verified, also appear to offer a possible explanation of the discrepancy between the findings in adrenalectomized mice by Szego and White on the one hand and those obtained in this and a number of other laboratories on the other hand.

8. Szego, C. M., and White, A., *Endocrinology*, 1949, v44, 150.

9. White, A., *Recent Progress in Hormone Research*, vIV, p. 153, Academic Press, Inc., New York, 1949.

10. Fry, E. G., *Endocrinology*, 1937, v21, 283.

11. McKay, E. M., and Barnes, R. H., *Am. J. Physiol.*, 1937, v118, 525.

12. Payne, R. W., *Endocrinology*, 1949, v45, 305.

TABLE 1.
Effect of Cortisone Pre-treatment on Liver Fat Response of Mice to Various Pituitary Preparations.

Exp. No.	Treatment‡	No.	Mean body wt, g	Liver wt. Mean ± σ* g/100 g BW	Liver wt. Change, %	Liver fat. Mean ± σ* mg/100 g BW	Liver fat. Change, %
1	Intact controls	41	20.8	5.53 ± .16		349 ± 8	
		Adrenalectomized.					
2	Untreated controls	7	21.8	5.00 ± .51	— 9	257 ± 18	—26
3	Cort. only	10	21.0	5.56 ± .25	+ 1	389 ± 14	+11
4	20 mg P18	5	24.9	4.33 ± .25	—22	281 ± 22	—19
5	Cort. + 20 mg P18	8	21.7	6.01 ± .24	+ 9	651 ± 43	+87
6	5 mg ACTH 71-2(50)c	6	23.3	5.36 ± .34	— 3	230 ± 17	—34
7	Cort. + 5 mg ACTH 71-2(50)c	8	24.8	5.52 ± .38	0	514 ± 24	+47
8†	5 mg ACTH 32-D	12	22.2	4.53 ± .16	— 6†	265 ± 13	—21†
9	Cort. + 5 mg ACTH 32-D	4	23.4	5.37 ± .21	— 3	623 ± 31	+79
10	Cort. + 5 mg GH R-95	8	23.7	5.87 ± .25	+ 6	638 ± 28	+83
11	Cort. + 5 mg GH 12PKR3	9	22.6	6.02 ± .14	+ 9	603 ± 38	+73
		Intact.					
12	Cort. only	10	24.5	6.12 ± .25	+11	386 ± 22	+10
13	5 mg GH #R-95	9	22.6	5.46 ± .28	— 1	354 ± 23	+ 1
14	Cort. + 5 mg GH #R-95	4	24.1	5.35 ± .31	— 3	552 ± 37	+58
15	5 mg GH #12PKR3	9	24.7	5.26 ± .22	— 5	338 ± 20	— 3
16	Cort. + 5 mg GH #12PKR3	4	23.9	5.35 ± .05	— 3	629 ± 26	+80

* The value for σ was calculated as $\sigma = \sqrt{\Sigma d^2/N(N-1)}$.

† The mice of this group were of another strain and therefore these results are compared to those of 36 control mice of that strain.

‡ All animals sacrificed at end of 7-hr fasting period. "Cort." indicates pre-treatment with 3.5 mg cortisone (suspension) in 7 equal subcutaneous doses during the 3½ days immediately preceding sacrifice. In adrenalectomized animals this treatment coincides with the period following adrenalectomy. Pituitary preparations were administered as a single subcutaneous injection at the start of the fasting period. The pituitary preparations used were the following:

P18—A crude, water soluble fraction from sheep pituitary glands.

ACTH 71-2(50)c—An Armour Laboratories preparation described as having 20% of the potency of the Armour ACTH Standard LA1A when assayed by the adrenal ascorbic acid depletion method. Substantially free of growth hormone.

ACTH 32-D—Armour preparation having 78% of the potency of LA1A. Negligible growth hormone content. 0.05 USP oxytoxic units and 0.1 pressor units per mg.

GH R-95—Armour preparation assaying 25 Evans growth units per mg.

GH 12PKR3—Approximately equivalent to the electrophoretically and ultracentrifugally homogeneous Armour standard growth hormone 22KR2. Essentially free of ACTH activity.

in 20% alcohol), the total dose thus being 3.5 mg during the 3½ days immediately preceding autopsy. In adrenalectomized animals this period coincided with the period of adrenalectomy, the treatment being initiated immediately post-operatively. Such animals also were given 1% sodium chloride to drink instead of water. Livers were removed immediately after sacrifice and total lipid content was determined by a modification of the method of Channon, Platt and Smith(13). Figures for liver lipid are presented as milligrams of liver "fat" per 100 g of body weight. However, it is emphasized that the results are essentially the same if liver lipid data are compared in terms of total quantity of fat per liver or as percent of fat in the wet liver.

Pertinent data are presented in Table I. It may be seen (Exp. 2) that a 3½ day post-adrenalectomy period results in a loss of liver fat as compared to the values obtained from intact controls. The loss (26%) suffered by the presently reported small group of adrenalectomized controls compares favorably with that noted in 2 larger groups of adrenalectomized mice of another strain. The liver fat content of the latter 2 groups (19 females and 17 males, respectively) was lower by 15 and 19%, respectively, than those of appropriate intact controls of the same strain.

The data further demonstrate (Exp. 3) that treatment with cortisone (1 mg per day for 3½ days) during the post-adrenalectomy interval prevents the loss of liver fat which otherwise follows adrenalectomy and even causes a very small increase. This increase is identical to that seen (Exp. 12) in intact mice similarly treated with cortisone. It may be added that we have obtained somewhat similar results by treatment with comparable doses of desoxycorticosterone acetate or of 11-dehydrocorticosterone. We have interpreted the small increases to be the result of non-specific supportive therapy rather than as a direct effect of the adrenocortical steroid on mobilization of fat.

Our uniform experience to date has been that pituitary extracts, regardless of variety, are unable to elicit an increase in liver fat on otherwise untreated adrenalectomized mice. One example of many such negative responses may be seen in the results obtained by treatment of adrenalectomized mice with the impure pituitary extract P18 (Exp. 4). It may be parenthetically added that the same preparation is quite active in *intact* mice, the same dose causing the liver fat content to increase by 44%.

The effect of cortisone treatment on the response of adrenalectomized mice to pituitary extract is shown in Exp. 5, Table I. In the animals pre-treated with cortisone the same pituitary extract discussed immediately above, (P18), produced an excellent response, the liver fat level being increased by 87% as compared to that of intact fasted control mice. These results are interpreted to indicate that (a) the fat-mobilizing action of the pituitary extract is not mediated by the adrenal cortex but (b) the extract is not able to cause fat mobilization unless the test animal has been supplied (from exogenous or endogenous sources) with adequate amounts of adrenocortical hormone.

Similar effects of pre-treatment with cortisone were obtained in adrenalectomized mice treated with ACTH. It is to be noted that neither of the two ACTH§ preparations (No. 32-D and 71-2(50)c) used in the presently reported experiments caused any increase in the liver fat content of otherwise untreated adrenalectomized mice (Exp. 6 and 8). It is of interest that these two ACTH preparations differed considerably in their potency as assayed by the adrenal ascorbic acid depletion method and, in intact mice, showed a proportional difference in ability to cause mobilization of fat to the liver. Thus, when assayed by depletion of ascorbic acid,§ preparation No. 32-D was approximately 4 times

13. Channon, H. J., Platt, A. P., and Smith, J. A. B., *Biochem. J.*, 1937, v31, 1736.

§ The ACTH and growth hormone preparations used in these experiments were generously furnished by the Armour Laboratories through the cooperation of Drs. John R. Mote and Irby Bunding. The potencies with regard to ACTH content (by adrenal ascorbic acid depletion) and to growth-promoting activity are those reported to the writers by the Armour Laboratories.

as active as No. 71-2(50)c. In intact mice, a 5 mg dose of ACTH 32-D caused a 78% increase in liver fat while a similar dose of No. 71-2(50)c yielded but a minimal response (8% increase). In adrenalectomized mice pre-treated with cortisone (Exp. 9 and 7), liver fat increases of 79 and 47%, respectively, were obtained with these two ACTH preparations. This result with preparation No. 71-2(50)c is particularly interesting in view of its inactivity in intact mice and is emphasized here because it demonstrates the sensitizing effect of cortisone pre-treatment particularly when used with pituitary preparations low in or completely lacking adrenocorticotrophic activity. These results not only confirm the conclusions drawn from the experiments with crude pituitary extract *(vide supra)* but also suggest that it is not the adrenocorticotrophic hormone, *per se,* which triggers the fat mobilization reaction.

Experiments with growth hormone preparations lead to similar conclusions. None of the 3 such preparations tested in this laboratory to date, when administered in doses up to 5 mg per mouse, has produced any increase in liver fat content *even in intact mice*[‡] (Exp. 13 and 15). Likewise, the 2 of these 3 growth hormone preparations which have been administered to otherwise untreated adrenalectomized mice have proven completely inactive in mobilizing fat to the liver. However, when administered to intact (Exp. 14 and 16) or adrenalectomized (Exp. 10 and 11) mice which had been pre-treated with cortisone, the two growth hormone preparations tested in this fashion both produced marked increases in liver fat content. It should be emphasized that these preparations are reported[§] by the manufacturer to be inhomogeneous but of a potency, measured by growth response, equal to that of pure growth hormone. It is also of considerable significance that, when assayed by the adrenal ascorbic acid depletion method, these preparations were found to contain very little, if any, demonstrable ACTH.

The results reported herewith, as well as those published by Payne(12), thus assume considerable significance with respect to the endocrine mechanism of fat mobilization. It has been shown in this and other laboratories that adrenalectomy completely abolishes the fat mobilizing response to administered pituitary preparations. This, plus the fact that many ACTH preparations are active in intact animals in this respect, has led to the impression that fat mobilization is a manifestation of ACTH activity mediated by the adrenal cortex. However, previous results(4-7) as well as the presently reported experiments throw considerable doubt on such an hypothesis. It now seems quite evident that a variety of pituitary preparations (crude extracts, ACTH or growth hormone) are able to cause mobilization of depot fat to the liver without the mediation or even the presence of the adrenal cortex provided the animal is adequately pretreated with cortisone (or perhaps other adrenal steroids).

Furthermore, the results here reported make it appear quite unlikely that fat-mobilization is a specific property of ACTH or of growth hormone. The fact that both ACTH and growth hormone, each substantially devoid of the other, are active in this respect (provided sufficient circulating cortisone has been available to the animal) and the fact(14) that the fat mobilizing potency of ACTH is not always proportional to the activity as measured by ascorbic acid depletion, both indicate that ACTH is not the causative factor.

‡ The explanation for the disagreement between these results and those reported from several other laboratories is not readily apparent. It may be pointed out, however, that the preparation used by Szego and White(8,9) was reported to be impure and an explanation may be forthcoming as indicated in footnote ‡. Also, it is perhaps significant to note that Payne(12) and Weil and Ross(15) used the same preparation (No. 3PKR3 of Armour Laboratories). Li *et al.*(16), on the other hand, used a presumably pure growth hormone of their own manufacture which was reported to cause an increase in liver fat content in hypophysectomized as well as in intact rats.

15. Weil, R., and Ross, S., *Endocrinology,* 1949, v45, 207.

16. Li, C. H., Simpson, M. E., and Evans, H. M., *Arch. Biochem.,* 1949, v23, 51.

14. Levin, L., and Farber, R. K., unpublished data.

This is substantiated by other data to be published elsewhere. However, it now becomes evident why certain ACTH preparations are quite active, of themselves, in causing fat mobilization in intact (but not in adrenalectomized) mice. If, as is indicated by the presently reported data, the two factors required to produce this response are an unidentified pituitary substance and 11-oxygenated steroid, then it is apparent that ACTH, slightly contaminated with an impurity of as yet unknown identity, can furnish both factors. The pituitary factor is thus supplied directly from exogenous sources while adrenocortical hormone is caused to be produced endogenously as a result of stimulation of the animal's own adrenals. An alternative possibility is that several pituitary hormones, including ACTH and growth hormone, are able to cause fat mobilization by a *direct action* but that ACTH, by virtue of its ability to cause secretion of adrenal steroids, is able to cause fat mobilization in the intact animal without the need for exogenously supplied 11-oxygenated steroid. Growth hormone, on the other hand, not being able to elicit increased adrenal cortical secretion would accordingly be inactive, even in intact animals, unless appropriate exogenous adrenocortical hormone is supplied.

Summary and conclusions. 1. Many, but not all, pituitary preparations produce an increase in liver fat content when administered to intact mice. This response is completely abolished by adrenalectomy.

2. Neither cortisone nor any other adrenocortical steroid tested to date is able, of itself, to cause a substantial increase in liver fat level of intact or of adrenalectomized mice. In the latter, however, the usual post-adrenalectomy loss of liver fat is prevented.

3. When pre-treated with adrenocortical hormone (cortisone), the ability of adrenalectomized mice to respond to pituitary hormone by mobilization of fat to the liver is equal to or greater than that of intact mice. Pituitary substances (growth hormone; weakly potent ACTH) inactive in either intact or adrenalectomized animals, became quite effective in mobilizing fat to the liver if the animals are pre-treated with adrenocortical hormone.

4. It is concluded that to mobilize fat from the depots to the liver the animal requires at least two factors, (1) adequate supplies of adrenocortical hormone (from either endogenous or exogenous sources) plus (2) a supply, either from its own anterior hypophysis or from exogenous sources, of the "triggering" pituitary factor.

5. The "triggering" action of the unidentified pituitary factor is not mediated via the adrenal cortex since it operates in the absence of the adrenal gland provided adequate amounts of exogenous cortisone are administered.

6. The possible identity of the "triggering" pituitary factor is discussed.

The authors are indebted to Miss Ilse Bauer for her kind assistance in performing the many adrenalectomies and necropsies.

Received June 19, 1950. P.S.E.B.M., 1950, v74.