

**Rate of Disappearance of Subcutaneous Vegetable Oil in Normal and Castrate Mice.\***

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Among the problems of fat metabolism so-called simple obesity has long been of interest to physicians. One point of view taken with regard to this condition considers it as merely another example of the operation of the law of conservation of energy. This attitude is, of course, unassailable, since it must be true that there is a disparity between expenditure of energy and food intake, if fat is to be stored in the subcutaneous tissues. At the same time, however, the phenomenon of obesity would appear to contain relationships of a biological nature which are less well defined. In the human subject, for example, obesity occurs with great frequency following certain disturbances of endocrine function, such as menopause, castration, or pituitary disease. No doubt in each instance there is a displacement of the equilibrium between caloric intake and output due to lowering of the total metabolism. But that this is the whole story is not so clear. The rate of deposition or utilization of depot fat and the type of mechanism called upon for its ultimate disposal both may be influenced by factors that are as yet poorly understood. The clinical association of obesity with abnormal endocrine function lends interest to this report of an observation which seems to indicate that even in normal animals there is a relation between sex and the rate of disappearance of an oil injected parenterally.

In the course of an experiment designed to study the effect of o-amino-azotoluol on the liver, the dye was dissolved in corn-oil ("Mazola") and injected subcutaneously into the backs of mice. The mice were of the closely inbred Bagg albino (C) strain and were all about 4 months old when first treated. They were 60 in number, 30 males and 30 virgin females, and were maintained on a diet of stock pellets which provides all of the necessary vitamins. As far as is known, they were normal animals. Each mouse received regularly each week for several months 0.1 ml of oil containing 2.5 mg of azotoluol. After about 3 months it was noted that there was a striking difference in the appearance of the males as compared

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with the females. The latter showed large humps on their backs representing encapsulated oil. The males, however, had failed to develop such swellings. This curious dissimilarity was quite unexpected, because the assumption had been made on the basis of general experience that such oils are physiologically almost inert and it seemed that these particular males had disposed of a surprisingly large quantity. Moreover, the difference was shown not only by a few animals but characterized virtually every one, so that it was possible by a glance at the back of any mouse to judge its sex with remarkable accuracy.

At first it was thought possible that this variation in behavior was dependent upon the presence of the biologically active carcinogen, and therefore the experiment was repeated using Mazola alone. Twenty male and 12 female Bagg albino mice about 3½ months of age were given 5 injections of 0.1 ml of oil at intervals of several days over a period of 3 weeks. One week after the last injection, 10 of the 12 females showed characteristic swellings of moderate size. Of the 20 males, only 6 presented any nodules and 4 of these were very small, while 14 had no visible or palpable collections of oil. At autopsy, direct examination of the involved areas bore out the preliminary impression that the amount of oil remaining in the females was significantly larger. Three weeks after the last injection, 4 males and 3 females that had been spared were examined again. The males, viewed from without, were negative but upon dissection showed very small droplets or blebs of oil distributed over an area of about 4 sq cm. The females, on the other hand, still presented externally visible humps, though these were, to be sure, smaller than before.

Following this experience, an attempt has been made to gain a first approximation to the amount of oil that may be recovered under these circumstances, and to determine also the effect of castration. For this experiment stock laboratory Swiss mice were used. The gonads were removed from 10 males and 10 females at the age of 22-23 days; *i. e.*, before puberty. Two months later these animals, together with groups of normal young adult males and females of about the same age, were started on a series of injections of Mazola. The oil was given in doses of 0.1 ml into the subcutaneous tissues of the left flank, biweekly until a total of 1 ml had been administered to all of the mice. Seven or 8 days after the last injection the animals were killed. (Attention should be called at this point to the fact that while the same gross dissimilarity between males and females appeared in this strain of mice, it was not as striking as in the Bagg albinos. Evidently in the Swiss mice the amount of oil

remaining unabsorbed is smaller in both sexes.) The blebs of oil, together with the adjacent overlying skin may be dissected away from the muscular sac containing the abdominal and thoracic viscera and spine without trouble. The oil was found to lie in thin capsules attached to subcutaneous tissue in area about the size of a quarter. This piece of skin was cut away, transferred to a Petri dish and extracted with anhydrous ether. The blebs were opened by scarification of the subcutaneous surface, and after a period of 5 minutes the ether was pipetted into a tube, centrifuged quickly at high speed to throw down hair and other solid particles, then finally transferred to a weighing bottle. Following evaporation and desiccation over  $\text{CaCl}_2$  the quantity of material remaining was determined by weight. The results are presented in Tables I and II.

In the first place the rate of disposal of injected oil is fairly rapid in

TABLE I.  
Ether-Soluble Material Recovered from Normal Young Adult Swiss Mice, mg.

|                               | Female                | Male           |
|-------------------------------|-----------------------|----------------|
| +++                           | 126                   | + 110          |
| +++                           | 192                   | + 113          |
| +++                           | 209                   | ++ 116         |
| +++                           | 261                   | + 152          |
| +++                           | 311                   | + 176          |
| +++                           | 329                   | ++ 190         |
| +++                           | 350                   | ++ 224         |
| Mean                          | $254 \pm 20.8$        | $154 \pm 11.3$ |
| $\sqrt{\frac{\sum d^2}{n-1}}$ | 81.7                  | 44.2           |
| Diff. of Means<br>x P.E.      | $100 \pm 23.7$<br>4.2 |                |

+ . + + indicates gross estimate of the relative amount of oil.

TABLE II.  
Ether-Soluble Material Recovered from Castrate Young Adult Swiss Mice, mg.

|                               | Female               | Male           |
|-------------------------------|----------------------|----------------|
| +                             | 167                  | + 152          |
| ++                            | 179                  | ++ 179         |
| ++                            | 205                  | + 232          |
| +                             | 217                  | ++ 233         |
| +                             | 239                  | ++ 249         |
| ++                            | 270                  | + 276          |
| ++                            | 294                  | ++ 300         |
| ++                            | 322                  | ++ 304         |
| ++                            | 380                  | + 308          |
| +                             | 487                  | ++ 383         |
| Mean                          | $276 \pm 21.1$       | $262 \pm 14.4$ |
| $\sqrt{\frac{\sum d^2}{n-1}}$ | 99.1                 | 67.6           |
| Diff. of Means<br>x P.E.      | $14 \pm 25.5$<br>.55 |                |

+ . + + indicates gross estimate of the relative amount of oil.

all the groups examined. For, even if it be assumed that all of the material recovered is vegetable oil, and if the specific gravity of the oil is about 0.9, then the maximum average recovery did not in any case exceed 30% of the quantity administered (900 mg). Actually, of course, some normal subcutaneous fat is extracted and contributes to the size of the figures given.

Secondly, there appears to be a significantly smaller average amount of ether-soluble material obtained from the normal males as compared to the normal females. Thirdly, after castration this sex differential is no longer apparent.

It is unnecessary to point out that the number of animals comprising this study is small and that there are errors inherent in the rather crude method of extraction. For instance, the figures given for the castrates are not to be strictly compared with the figures for the normal animals. The larger fat pad which develops after castration would, in any case, increase the quantity of ether-soluble materials obtained, regardless of the relative amounts of oil present. However, these data represent only the results of a preliminary effort to measure the rate of disposal of the oil, and do provide at least an approximation of the magnitudes involved.

In support of the idea that these observations reflect a relationship between sex hormones and intermediate metabolism of fat, mention may be made of so-called lipemia in birds. The subject has been recently reviewed<sup>1</sup> and need be examined here quite briefly. Estrogens exert a specific action on the blood fat of both male and female birds, producing enormous increases in the lipid content of the blood. Neutral fats, as well as phospholipids and cholesterol, share in the increase. Androgens do not have this effect. Riddle remarks, "Despite great gaps in our knowledge, particularly in the case of mammals, it is of much importance that estrogenic hormones have now been shown to have surprising ability to increase the blood fat in birds."<sup>1</sup> The method used in the experiments described above may prove of value for further exploration of the problem.

*Summary.* 1. Vegetable oil administered parenterally to mice is not physiologically inert but disappears rather rapidly from the site of injection. 2. The rate of disappearance of oil appears to vary to some extent in different breeds of mice. 3. The rate of disappearance of oil in both of two different strains of mice appears to be higher in normal young adult males than in normal females of the same age. 4. In a small series of mice, castration before puberty abolished this sex differential.

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<sup>1</sup> Riddle, O., *Ann. Rev. Phys.*, 1941, **3**, 588.