Effect of Ovariectomy on Blood Concentration of Orally Administered Triolein I^{131} in Bitches on Low and Moderate Fat Intake.* (27888)

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The use of labeled triolein as an agent for diagnosis of various malabsorptive conditions has been well established(1-4). More recently, high rates and magnitudes of triolein I^{131} appearance in the circulation have been associated with the presence of vascular disease in patients(5-8). Higher than normal peaks of blood radioactivity following oral administration of triolein I^{131} have been reported in cases of obesity(6) and idiopathic hyperlipemia(9). There is evidence that the differences in atherosclerotics persist only during the earlier ages(7). Because of the established association between human vascular disease and loss of ovarian function(10, 11,12), measurements were made of blood radioactivity following oral administration of triolein I^{131} to castrate bitches. The effect of dietary fat level was also observed.

Materials and methods. In this experiment triolein I^{131}† was administered *per os* to intact and ovariectomized beagle females under 2 levels of dietary intake. The percentage of administered radioisotope present in the circulation at intervals after administration was determined. No effort was made to fractionate the labeled material into trichloracetic acid precipitable or aqueous fractions.

Eleven beagle females were examined. All were young mature animals and 6 had been ovariectomized before puberty. Blood volume was measured by dye dilution(13) shortly before the first experiment. In the expectation that a given animal would deviate consistently from the average under similar conditions and since body weight was stable throughout the experimental period, the values were assumed valid. Prior to the first experiment the animals were maintained on a diet consisting, on a dry weight basis, of 60 lb dry dog food‡ containing less than 1% fat, 20 lb meat protein, 8 lb cottonseed oil, and 9 lb whole eggs. These components were wetted, thoroughly mixed and frozen for storage. To establish accommodation to low fat intake, subjects were maintained for 6 weeks on dry dog food. These diets were constantly available until administration of radioactive fat.

Lugol's solution was not administered in preparation for either experiment.

For use in the first experiment, triolein I^{131} was diluted in cottonseed oil to an activity of approximately 35 μc/10 ml. Ten milliliters were carefully measured, administered by a stomach tube to each animal, and washed into the stomach. A similar exactly measured fraction was prepared and diluted to 1000 ml. Three ml of this standard were placed in a plastic counting tube and counted to provide a reference from which the percentage of activity in circulation could be calculated. For the second experiment, triolein I^{131} was diluted in a gelatin capsule which was then enclosed in a second gelatin capsule. Uniformity of dosage was checked by measuring the radioactivity of each capsule before administration. A sample capsule prepared identically was crushed and brought to a volume of 1000 ml to establish a reference standard. Capsules were administered *per os* and all were ingested without breakage.

Samples were drawn at 40, 80, 120, 160, and 280 minutes, and at 24 hours in the first experiment. In the second experiment, during low fat intake, the schedule was similar except that additional samples were drawn at 20, 60, and 100 minutes. Three ml of each sample were placed in a counting tube and radioactivity was measured by a NaI crystal well scintillation manual spectrometer system

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† Raolein, Abbott Laboratories.

‡ F/D, Hill Packing Co.

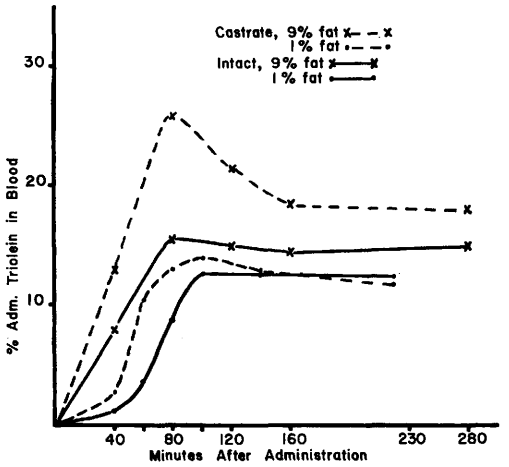

FIG. 1. Percent triolein I^{131} in circulation after oral administration.

utilizing differential counting at the I^{131} photopeak.

Results and discussion. In these experiments a distinctly higher peak in blood concentration of isotope occurred in the castrate bitches during a period of relatively high fat intake (Fig. 1). This could be due to a difference in utilization or deposition, or to a difference in absorption.

When uptake of labeled fat was measured during an extended low fat diet ovariectomized animals again produced higher blood radioactivity curves than did intact controls (Fig. 1). Under these conditions, however, the mean differences were less, and peak times and magnitudes were more erratic. We believe that diet during the period prior to clinical use of this test may influence the validity of the results. The plateau following peak value was approximately the same in both control and castrate bitches.

The lower, erratic peaks occurring during low fat intake are difficult to explain. Janssen *et al.*(14) administered labeled triolein directly and in small volume into the duodenum and found significant impairment of absorption. Oral lipid prefeeding abolished the defect, leading to the conclusion that insufficiency of digestive enzyme and bile was responsible. The assumption can be made in our study that adequate lipolytic and emulsifying activity is stimulated by intestinal content regardless of fat content.

The relatively short time course in dogs is of interest. In normal man an absorption peak may be expected near the sixth hour (5), but the peak time for both groups of animals in these experiments was about 80 minutes. No correlation was found between blood radioactivity levels and total body fat as measured in the course of other work, using a modification of the method of Lesser, Blumberg and Steele(15,16).

One attempt was made to use commercially prepared capsules, which are calibrated and very convenient. Under the conditions of these experiments, however, absorption was exceedingly slow; blood concentrations seldom exceeded 1% of the administered dose. In most animals the isotope was resident in the gut for 3 days, with no rise in blood radioactivity.

Summary. Triolein I^{131} was administered to castrate and intact female beagles maintained on 2 levels of dietary fat. Castrate bitches were found to have a greater maximum percentage of administered radioactivity in circulation than intact females. Curves representing blood radioactivity plotted against time were similar after castration to those of some conditions in the human in which deranged lipid metabolism is implicated. Curves produced under conditions of low availability of dietary fat become erratic and presented only slightly different mean peak values when intact and castrate animals were compared.

1. Stanley, M. M., Thannhauser, S. J., *J. Lab. Clin. Med.*, 1949, v34, 1634.

2. Benes, P., Wenger, J., Kirsner, J. B., *Gastroenterology*, 1957, v32, 1.

3. McKenna, R. D., Bourne, R. H., Matzko, A., *ibid.*, 1957, v32, 17.

4. Shingleton, W. W., Baylin, G. J., Isley, J. K., Sanders, A. P., Ruffin, J. M., *ibid.*, 1957, v32, 28.

5. Berkowitz, D., Sklaroff, D., Woldrow, A., Jacobs, A. k G., Likoff, W., *Ann. Int. Med.*, 1959, v50, 247.

6. Berkowitz, D., *Am. J. Clin. Med.*, 1960, v8, 327.

7. Edelman, J., Sandberg, H. Dickstein, E. R., *Am. J. Cardiol.*, 1961, v7, 676.

8. Seller, R. H., Brachfeld, J., Sandberg, H., Bellet, S., *Am. J. Med.*, 1959, v27, 23.

9. Meng H. C., *Am. J. Clin. Nutr.*, 1961, v9, 68.

10. Oliver, M. F., Boyd, G. S., *Lancet*, 1959, v2, 690.

11. Robinson, R. W., Higano, N., Cohen, W. D., *Arch. Int. Med.*, 1957, v100, 739.
12. ———, *ibid.*, 1961, v104, 908.
13. Chinard, F. P., *Methods in Med. Research*, 1951, v4, 38.
14. Janssen, B., Tyor, M. P., Owen, E. E., Ruffin, J. M., *Gastroenterol.*, 1960, v38, 211.
15. Lesser, G. T., Blumberg, A. G., Steele, J. M., *Am. J. Physiol.*, 1952, v169, 545.
16. ———, in *Biochemical Problems of Lipids*, G. Popjak, and E. LeBreton, Eds., Interscience Publishers, New York, 1956, 373.

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A Tumor Inhibitor in *Lampteromyces japonica.** (27889)

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In the fall of 1958, the senior author working in the laboratory of Dr. Ayao Yamamoto, Institute for Infectious Diseases, University of Tokyo, cooperated with Drs. Yamamoto, Ogata, Komatsu and Nakazama in studying the effect of saline extracts of *Lampteromyces japonica* (L.J.) on Ehrlich mouse tumors and Sarcoma 180. This work included *in vivo* and *in vitro* studies with findings of some anti-tumor activity. Lucas *et al.*(1,2) have demonstrated tumor inhibition by extracts of the fungus genus Calvatia and Holobasidiomycetes.

It seemed desirable to extend these preliminary observations and to determine the effect of fractions of L.J. on the Ehrlich mouse tumor and on human tumor cells growing in tissue culture.

Materials and methods. The tumor-inhibiting original extract was prepared from dried *Lampteromyces japonica,* the only species of this basidiomycete. The original samples were collected in Japan in 1959.

Ten g of dried material, ground in a 20-mesh Wiley Mill, were extracted with 100 ml of saline (0.85% NaCl) for 24 hours at 5°C with continuous shaking. The saline extract was then spun at 500 *g* for 20 minutes. The residue was reextracted with 100 ml of saline. Part of the pooled supernatants was dialyzed at 5°C against running tap water for 48 hours and then against distilled water for 3 hours. This saline extract was designated L.J., original extract. To the remainder of the pooled supernatant at a pH of 5.5-6.0, was added cold (5°C) ethanol to a final concentration of 30%. This was kept at 5°C overnight and then centrifuged at 5000 *g* for 30 minutes in an anglehead centrifuge. The 30% alcohol precipitate was dialyzed and lyophilized. This was designated as fraction No. 2. To the 30% ethanol supernatant, cold ethanol was added to a final concentration of 70%, then kept overnight at 5°C. This material was then centrifuged at 5000 *g* for 30 minutes and the supernatant was decanted, dialyzed and lyophilized, as was the precipitate. The latter was labeled fraction No. 3, and the lyophilized supernate fraction No. 4.

In vitro *inactivation of Ehrlich tumor cells.* Seven-day Ehrlich ascites tumor cells were washed free of red blood cells with 0.1% gelatin saline and made up to a 4% suspension by volume. Equal volumes of 1% and 0.1% initial concentrations of the L.J. fractions in saline were added to aliquots of the tumor cell suspension. After the mixtures had been incubated at 37°C for 30 minutes, 5 white mice (male, 18-20 g random bred Swiss) per test group were injected subcutaneously in the inguinal region, with each animal receiving 0.2 ml of the tumor cell suspension. This inoculum represents about 1.5 million cells, which gave 90-100% takes in controls. These mice were observed twice weekly for 3 weeks, and the development of tumors recorded.

In vivo *animal bioassay inactivation.* Mice

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