The presence of actively growing Walker carcinoma 256 in rats has been shown to affect various non-lipid components of the organs of the tumor-bearing host (1) (2). The present work was undertaken to investigate quantitative changes in carcass lipids and in lipids and steroids in the blood plasma and adrenals of rats bearing Walker carcinoma 256.

MATERIALS AND METHODS

Small pieces of Walker 256 tumor were transplanted subcutaneously into the groin of young male rats. When the tumors comprised from 11 to 48 per cent of the total body weight the animals were sacrificed, tissues removed and the lipids of carcass, blood plasma, and adrenals determined. Normal rats and rats in which the tumor did not grow after transplantation (non-take rats) served as controls.

Rats to be used for determination of carcass lipids were on a diet containing 1.3 per cent fat (3) consisting of coconut oil or cod-liver oil. The remainder of the animals were on a diet of Purina fox chow.

Carcass lipids.—The rats were weighed and killed by decapitation; the tumors were removed and weighed and the carcasses placed in a 30 per cent solution of potassium hydroxide in 95 per cent ethanol. Tumors were dissolved separately in the alcoholic potassium hydroxide solution. After several days the small undissolved residue of bones from each carcass was powdered with a stirring rod and the whole suspension made to 500 ml. with distilled water. Aliquots were evaporated on the steam bath, acidified to Congo red with 3N hydrochloric acid and the lipids extracted with a 6:1 mixture of petroleum ether-chloroform. The lipid extract was washed once with 40 per cent ethanol, after which the extract was drawn off into a small weighed Erlenmeyer flask, the solvent removed, and the lipid residue dried to constant weight. The lipids of the tumors were extracted and treated in the same manner.

Plasma lipids.—Blood obtained by heart puncture under ether anesthesia was citrated and centrifuged. To the plasma samples (2 to 4 ml.) were added distilled water to make 4 ml. and sufficient potassium hydroxide to make the solution approximately 8 per cent. The contents of the tubes were mixed and the tubes left on the steam bath overnight (temperature about 85° C.). The solution was then acidified with 10N sulphuric acid and extracted for 5 minutes with 3 to 4 volumes of 7:1 petroleum ether-chloroform by use of a shaking machine. The tubes were centrifuged and the lipid extract drawn off. The extraction process was repeated twice after which the extracts were combined and made to a volume of 25 ml. Analysis for total lipid was carried out by a method previously described (4).

Adrenal lipids.—Adrenals were removed from the rats, carefully freed from adhering fat and connective tissue, weighed to the nearest 0.5 mg. and placed in 15 ml. centrifuge tubes. To the tubes were added 4 ml. of distilled water and sufficient potassium hydroxide to make the solution approxi-
mately 8 per cent. The adrenal suspension was stirred from time to time to dissolve the tissue after which the tubes were left on the steam bath overnight (temperature about 85° C.). The alkaline digests of the adrenals were then extracted with the petroleum ether-chloroform solvent as described under "blood lipids" and the combined extracts made to a volume of 25 ml. This solution of lipids which will be referred to as the "alkaline extract" was found to contain about 85 per cent of the steroid (Liebermann-Burchard reaction).

The residual alkaline water solution was acidified with 10N sulphuric acid, and extraction with the petroleum ether-chloroform solvent was carried out as described above. This "acid extract" contained mainly the fatty acids from the glycerides including lecithin and cephalin. The steroid esters were not appreciably hydrolyzed by the alkali and therefore appeared in the "alkaline extract." These extracts were analyzed for total lipid and cholesterol by the method previously published (4).

RESULTS

Carcass lipids.—In Figure 1 the per cent lipid in the combined carcass and tumor of 17 tumor-bearing rats is plotted against the tumor as per cent of the total body weight. The per cent lipid varied inversely with the size of the tumor and was independent of the kind of fat in the diet. Since the average per cent lipid was 9.1 with an average deviation of ± 1.7 in the carcasses of 14 "non-take" rats on the same diets, the presence of actively growing tumor decreased the amount of total lipid in the animal.

Total lipid of blood plasma.—Total lipid values on samples of plasma from 44 normal rats averaged 161 mg. per cent with a median value of 166 mg. per cent; range 60 to 245. Values for total lipid of plasma of 52 rats bearing tumors were elevated above normal in most instances. Because of the wide range in values (143 to 790 mg. per cent) and the great variation in size of the tumors (13 to 48 per cent of total body weight) an average would be meaningless. The median value was 325 mg. per cent. Frequency distribution curves for total lipid values of plasma of the normal and tumor-bearing group are shown in Figure 2. It can be seen that the frequency distribution curve for the rats without tumor is essentially normal while that for the tumor-bearing rats is irregularly displaced in the direction of values for total lipid higher than normal.

Cholesterol of blood plasma.—Values for cholesterol of plasma of normal rats averaged 60 mg. per cent with a range of 18 to 84 and a median value of 62. The range in values for plasma cholesterol in the tumor-bearing group was greater (31 to 156) but the median value of 66 mg. per cent was close to the normal. Therefore the elevated values for total lipid of plasma in rats with tumors presumably is due to increased values for total fatty acid of the blood plasma or possibly to increases in steroids other than cholesterol.

Lipids of the adrenals.—The lipid content of the adrenals in normal rats and in tumor-bearing rats grouped according to tumor percentage of the total body weight is shown in Table 1. In rats with large, actively growing tumors constituting over
30 per cent of the total body weight the average per cent total steroids in the adrenals decreased to about one-third of the normal value of 5.3 per cent. In animals with smaller tumors the total steroids of the adrenals decreased but to a lesser extent.

The average values for cholesterol of the adrenals were below normal in all tumor-bearing groups. The decrease in this steroid varied directly with the tumor percentage of the total body weight.

Values for fatty acids had decreased slightly in the adrenals of rats with large tumors but had increased in the adrenals of rats with smaller tumors.

**DISCUSSION**

The median values for total lipid of the blood plasma in the four classes of tumor-bearing rats showed an increase as the tumor percentage of total body weight increased. In all probability no great significance can be attached to this apparent relationship because of the wide range of values in each group. The increase in plasma lipids in rats with tumors is probably due to increased mobilization from the fat stores which have been greatly depleted in rats with large tumors (Fig. 1). The observation by Mider (5) that rats growing Walker carcinoma 256 lose significantly more total lipid than their pair-fed controls indicates a greater utilization of fat for energy in tumor-bearing than in normal rats. The effect of the various hormones especially those from the pituitary gland on the mobilization of fat is considerable and the possibility of a hormonal explanation for the lipemia must be kept in mind.

By the use of histologic techniques Dalton and Peters (6) observed a decrease in stainable lipid in the adrenals of tumor-bearing mice. Aoki (7) reported a chemical decrease in the concentration of fatty acids, cholesterol, cholesterol esters, and phospholipids in the adrenals of rats bearing a hepatoma.

In our rats growing Walker tumor 256, depletion of adrenal steroids was characteristic of the animals with large tumors and may be explained in the same way as the alarm reaction of Selye (8) or the exhaustion discussed by Long (9). The decrease in the specific steroid, cholesterol, is in line with the current belief that cholesterol is a precursor of the adrenal steroids (8). An inverse relation between the steroid and fat content of the adrenals appears to exist. As the steroids were depleted their place was at first taken by fat and as the animals became more emaciated the fat was used up.

Hyperplasia of the adrenals was also characteristic of the animals with large tumors. The adrenals in these animals sometimes weighed as much as three times the normal. Such enlargement may be interpreted as an effort on the part of the organism to compensate for the deficiency created by the exhaustion. The result is that the amount of steroid per 100 gm. of rat plus tumor was often normal although the percentage content of the adrenal was much below the normal average.

**TABLE 1**

| Lipid Content of the Adrenals in Normal and Tumor-Bearing Rats (Gm./100 Gm. Adrenal) |
|----------------------------------|----------------------------------|----------------------------------|
| Total Steroids | Fatty Acids | Total Lipid |
| Rat Group | No. rats | Av. | dev. | No. rats | Av. | dev. | No. rats | Av. | dev. |
| Normal | 50 | 5.3 | ±1.2 | 49 | 2.7 | ±0.9 | 50 | 7.5 | ±1.1 |
| Tumor: per cent of total body wt. |
| 10-19 | 28 | 3.1 | 1.0 | 24 | 1.7 | 1.0 | 25 | 9.8 | 3.0 |
| 20-29 | 20 | 2.1 | 0.7 | 19 | 1.2 | 0.7 | 20 | 9.6 | 2.6 |
| 30-39 | 12 | 1.5 | 0.5 | 12 | 1.0 | 0.3 | 11 | 6.9 | 2.4 |
| 40-48 | 14 | 1.9 | 0.8 | 14 | 1.2 | 0.6 | 13 | 5.1 | 2.2 |

In both adrenals and blood, the range of lipid values was great which was probably due to individual variations in the response of the animals to the presence and growth of the tumors.

**SUMMARY**

In rats bearing Walker carcinoma 256:

1. Carcass lipids varied inversely with the size of the tumor.
2. Blood lipids, chiefly fatty acids, were increased in most cases; values several times normal were frequently found.
3. In the adrenals: (a) Hyperplasia was characteristic of the animals with large tumors; adrenals frequently weighed as much as three times normal. (b) The average per cent total steroid decreased to about one-third of the normal value in rats with large tumors. (c) The steroids were replaced to some extent by fat.

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Lipids of the Carcass, Blood Plasma, and Adrenals of the Rat in Cancer

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