Mice can be actively sensitized to either HSA or BSA with multiple injections. Three interperitoneal injections of HSA (10 mg/injection, 7 days between injections) resulted in 50% of mice being anaphylactically shocked when challenged intravenously 21 days following the first injection(3). Mice were not anaphylactically shocked when challenged 7 or 21 days following a single intraperitoneal injection of HSA (30 mg)(3). Four subcutaneous injections of BSA (4 mg/injection) administered on an every-other-day schedule resulted in approximately 75% of mice being anaphylactically shocked when challenged 14 days following first injection(4). A single subcutaneous injection of BSA (16 mg) results in only 1 or 2% being anaphylactically shocked when challenged on 14th day. Thus in comparison, a single intravenous injection of HSA or BSA (about 0.05 mg) plus its specific antiserum is as effective in actively sensitizing mice to serum albumin as multiple (interperitoneal or subcutaneous) injection of serum albumin alone.

Several studies have been reported in which antigen and antibody were combined and active sensitization measured (*e.g.*, 5,6,7). None of these, of which we are aware, revealed the marked differences in response here described (HSA alone *vs.* HSA plus antiserum). In fact, the opposite effect has been reported(7). Thus ovalbumin-rabbit antiovalbumin precipitates were incorporated in adjuvant and injected into foot pads of guinea pigs. These precipitates were formed in both antigen and antibody excess. Other guinea pigs were similarly injected with an equal amount of "free" ovalbumin. When these animals were challenged with ovalbumin 2 to 3 weeks later, it was found that a higher degree of sensitivity had been achieved with "free" ovalbumin than with the immune precipitates.

Summary. Mice were injected intravenously with a solution of serum albumin (human and bovine) plus specific rabbit antiserum in antigen excess. The antibody response to serum albumin was tested and compared to that obtained in mice injected intravenously with serum albumin alone or with albumin and normal rabbit serum. The results show that antibody response elicited with serum albumin plus specific rabbit antiserum is significantly greater than that elicited using serum albumin alone or with normal rabbit serum.

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Level of 3-Methylcholanthrene in Mammary Glands of Rats after Intragastric Instillation of Carcinogen.* (25343)

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Development of mammary adenocarcinoma in rats following oral administration of 3-methylcholanthrene has been reported(1,2). These observations also indicated that induced mammary cancers were hormone-dependent. Our studies have further demonstrated that, in rats, steroid hormones promote mammary carcinogenesis by 3-methylcholanthrene(3). In earlier reports by Shay *et al.*(1) and later by Huggins *et al.*(2) and by Dao *et al.*(3),

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TABLE I. Levels of 3-Methylcholanthrene in Tissues of 50-Day-Old Virgin Female Rats Fed the Hydrocarbon.

Tissue	Level of 3-methylcholanthrene (μg/g tissue)
Breast, rat 1	15
Fat, "	70
Breast, rat 2	12
Fat, "	17
Breast, rat 3	24
Fat, "	38
Brain, pooled	3.5
Lung, "	2.1
Kidney, "	1.1
Thymus, "	1.4
Liver, "	.3
Muscle, "	.04
Heart, "	*
Spleen, "	*
Uterus, "	*
Ovaries, "	*
Adrenals, "	*
Pituitaries, "	*

* Not found.

Total content of 3-methylcholanthrene was less than 0.1 μg in pooled tissues of 3 rats.

mammary tumors were the only neoplasms observed in rats fed with 3-methylcholanthrene. Selective response of mammary tissue to orally administered 3-methylcholanthrene cannot be explained fully, since the mechanism of carcinogenesis is not known. However, reports(4,5,6,7,8,9,10) have demonstrated selective concentration of administered polycyclic carcinogens in breast and fat, and in milk of lactating females of various animals. The earlier reports, however, were concerned primarily with qualitative demonstration of compounds in tissues because of lack of instruments for measuring concentrations of the polycyclic hydrocarbons in the presence of tissue impurities. With recent development of spectrophotofluorometers, it has been possible to determine distribution of 3-methylcholanthrene in tissues on a quantitative basis and to carry out detailed clearance studies in specific tissues. Because of specific effects of 3-methylcholanthrene upon rat breast, results of preliminary studies are of general interest.

Method. 3-methylcholanthrene was fed through a stomach tube, a firm rubber (#10 F) catheter, 5 to 6 cm long, attached to the adapter of a syringe. In the first experiment, a single feeding of 30 mg of 3-methylcholanthrene in 1 ml of sesame oil was given to 3 virgin Sprague-Dawley Albino female rats at 50 days of age and a single feeding of 10 mg/ml sesame oil to 3 lactating rats 7 days after parturition. Twenty-four hours later, animals were sacrificed; tissues as listed in Table I were removed for analysis. In the study in which the content of 3-methylcholanthrene in milk was examined, the milk was obtained from the stomach of offspring of females fed 10 mg of 3-methylcholanthrene 24 hours earlier. In a second experiment, breast and abdominal and retroperitoneal fat were removed from animals that had received one single feeding of 30 mg of 3-methylcholanthrene and had been sacrificed at intervals of 1, 2, 3, 4, and 8 days. All dissecting instruments and glassware were specially cleaned to avoid contamination with methylcholanthrene. Tissues were weighed, minced, and treated with equal volumes (ml/g tissue) of 95% ethanol and 10% aqueous potassium hydroxide. They were heated under reflux for 6 to 8 hours, then extracted twice with benzene. The pooled benzene fractions were examined in the Aminco-Bowman Spectrophotofluorometer using either 295 mμ or 355 mμ excitation light and measuring the 410 mμ emission peak. Crude extracts of breast, fat, milk, brain, lung, and thymus could be analyzed directly (Fig. 1a, 1b). Other tissues contained low ratios of 3-methylcholanthrene to interfering substances and therefore had to be purified further. Purification was achieved by removal of benzene from the extract, which was then taken up in cyclohexane and passed through a silica gel column. The column was washed with cyclohexane, then 3-methylcholanthrene was eluted with benzene. With this procedure, 3-methylcholanthrene levels could be determined in liver and kidney. At time of analysis, each sample was read, diluted, and read again, to ascertain that the photometer response was proportional to concentration. A reference standard was read before and after each sample. Recovery of known amounts of 3-methylcholanthrene from tissues was between 85 and 95%, using the complete procedure.

Results show that 24 hours after oral administration of 3-methylcholanthrene to rats,

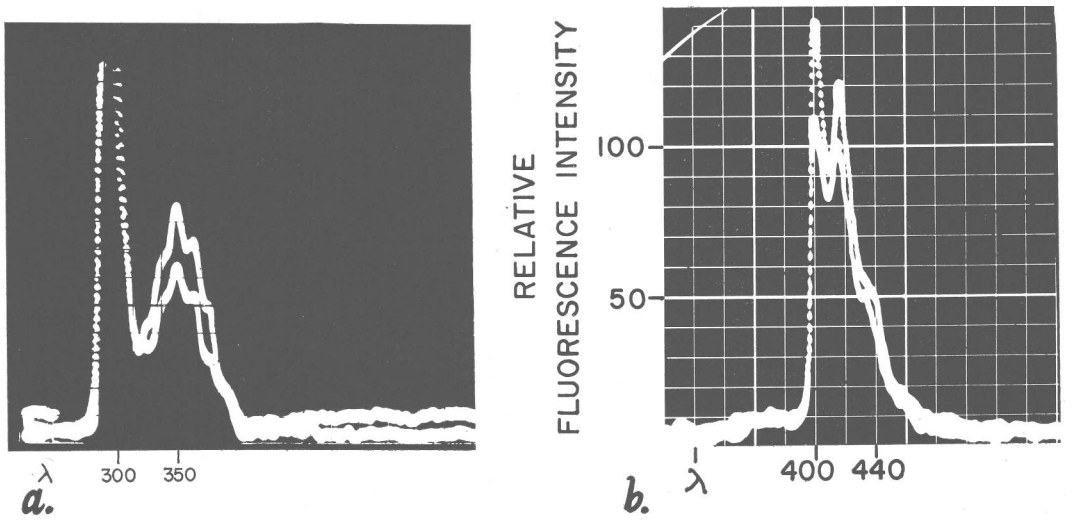

FIG. 1a. Excitation spectrum. Upper curve, 3-methylcholanthrene, 1 γ/ml; lower curve, crude extract of hydrolyzed milk curd. Emitted light = 410 mμ.

FIG. 1b. Emission spectrum. Upper curve, 3-methylcholanthrene; lower curve, crude extract of hydrolyzed milk curd. Excitation light = 295 mμ.

the aromatic hydrocarbon is found mainly in fatty and breast tissues (Table I) while levels in other tissues are either very small or insignificant. It also seems evident that levels of 3-methylcholanthrene are higher in fat than those in breast tissues. However, in the lactating breast, the level of 3-methylcholanthrene is reversed, higher in breast tissue and lower in fat. Milk curd from fetuses, of the stomach contained high concentration of 3-methylcholanthrene after one single feeding of the carcinogen.

Results of second experiment show that the level of 3-methylcholanthrene decreases rapidly in the breast and fat within 72 hours, but a significant amount of 3-methylcholanthrene remains in both tissues even at end of 8 days (Fig. 2). Rate of clearance of ingested 3-methylcholanthrene is about the same in both fat and breast tissues.

Discussion. It is evident from our results that both fat and breast tissues contain high concentrations of 3-methylcholanthrene after a single feeding of the hydrocarbon. Breast tissue from rats contained a large amount of adipose tissue, which may have accounted for much of the 3-methylcholanthrene. However, the high levels of 3-methylcholanthrene in breasts as well as the high content of the carcinogen in milk of lactating rats suggest that 3-methylcholanthrene was present in glandular cells of mammary glands since it is from these cells the milk is produced. Furthermore, the fact that the 3-methylcholanthrene in the lactating breast tissue is higher than that of the fat is indicative of the presence of carcinogen in cells of a hyperfunctioning mammary gland (Table II).

It is believed that carcinogens take a considerable time to manifest their effects. Dur-

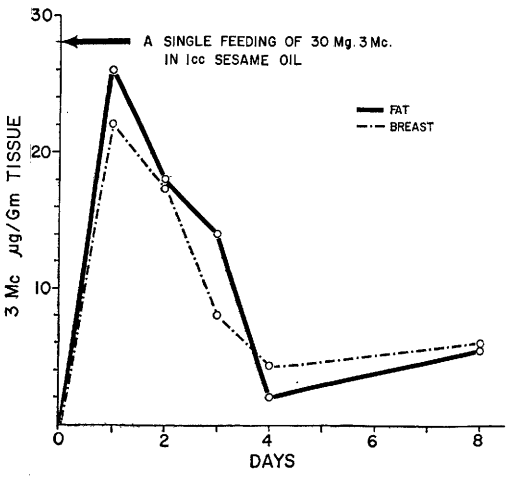

FIG. 2. Clearance of 3-methylcholanthrene from the breast and fat following a single feeding of 30 mg of the carcinogen in 50-day-old female Sprague-Dawley rat. Note persistence of 3-methylcholanthrene in these tissues 8 days following single feeding. 3 Mc = 3-methylcholanthrene.

TABLE II. Levels of 3-Methylcholanthrene in Breast and Fat of Virgin Rats and in Milk, Breast and Fat of Lactating Rats. One single feeding of 3-methylcholanthrene of 10 mg/cc sesame oil was given and animals were sacrificed 24 hr later.

	Tissues	Wt (g)	3-methylcholanthrene (μg/g tissue)
Virgin rats 50 days old I	Breast	2.6	6.5
	Fat	.9	7.1
II	Breast	2.1	6.4
	Fat	.9	10.9
Lactating rat I (7 days postpartum)	Breast	11.5	17
	Fat	1.7	11
	Milk (fetus 1)		23
	(" 2)		13

ing the latent period, further exposure of cells to the carcinogen produces enhanced effects. Our experiment shows that 3-methylcholanthrene is removed from the breast with passage of time, but a significant amount remains in these tissues for a prolonged period following a single feeding. The possibility that persistence of 3-methylcholanthrene in mammary glands may be important for neoplastic transformation has been clearly shown by Huggins' experiment in which a single feeding of 20 mg of 3-methylcholanthrene elicited mammary cancer in rats after a long latent period(2).

Thus, in chemical carcinogenesis of the rat breast, 2 factors seem to be involved: first, concentration of administered carcinogen in the target tissues; and second, persistence of the carcinogen in these tissues. Perhaps the adipose tissue of mammary gland functions as a storage depot of the carcinogen. Proximity of adipose tissue to the mammary gland tissue facilitates further exposure of mammary epithelial cells to the carcinogen, thus augmenting the neoplastic transformation. The vehicle, sesame oil, may also play an important role in transportation of fat-soluble carcinogens to breast and fat. Studies with aqueous suspensions of 3-methylcholanthrene are now being undertaken.

Summary. 3-methylcholanthrene in a single dose of 10 mg/ml sesame oil when fed to rats through a gastric tube, concentrated in the breast and fat of virgin female rats and in milk and breast of lactating rats. The level of 3-methylcholanthrene in other tissues was either very small or insignificant. It was further demonstrated that 3-methylcholanthrene persisted in a significant amount in these tissues at the end of 8 days following a single feeding of the carcinogen.

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