almost entirely accounted for by the increase in the hexosamine content of the beta and gamma globulin fractions. The normal distribution of hexosamine in rat plasma is as follows: Albumin 5%, alpha globulins 67%, beta globulins 19%, and gamma globulin 9%. The richest fraction is alpha-1 globulin which contains approximately 4.4% hexosamine. The other globulins contain less and albumin contains only 0.30%.

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Stimulation of Adenine Uptake into Mouse Lung and Liver *in vitro* By Tumor Extracts.* (21923)

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Kelly and Jones(1) observed that repeated injections of tissue mashes into normal animals bring about changes in the nucleic acid turnover of the spleen and liver. Our studies (2) have indicated that single injections of a tumor homogenate into normal mice alter the incorporation of adenine-8-carbon^{14} (C^{14}) into the desoxyribonucleic acid (DNA) of several tissues. Fractions obtained from tumor homogenates by centrifugation or pH adjustment were tested for the presence of the factor(s) responsible for this effect by injection into normal mice simultaneously with adenine-8-C^{14} solution. Increase of lung DNA C^{14} activity in animals which had received the tumor preparations was taken as an indication that the factor(s) was present in the preparation. Goldwasser(3) reported that an *in vitro* system consisting of rat liver slices or of cell-free homogenates of pigeon liver incorporated adenine into ribonucleic acid but not into DNA. In simplifying the assay procedure for the tumor factor(s), the authors have developed an *in vitro* system in which significant increases in DNA C^{14} radioactivity

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after proper incubation with adenine-8-C^{14} have been observed.

Methods. The *in vitro* system consisted of a tissue mince prepared from the lungs and livers of normal 9- to 11-week-old C57 black mice.§ The lungs and livers were rapidly excised, weighed, placed on a watch glass, and minced together by scissors and scalpel until a finely divided mince of the combined tissues was obtained. Approximately equal portions of the mince were placed in wide mouth, 4-inch centrifuge tubes. Three to 5 ml of Ringer's solution fortified with glucose and buffered to pH 7.4 with a .01 ionic strength phosphate buffer was added to each tube. The tubes were maintained at 37°C in a water bath, and the contents of the tubes were continuously aerated by forcing air through long capillaries opening near the bottom of the solution in each tube. The same amount of adenine-8-C^{14} was then added to each tube, approximately 0.5 μc. Tumor homogenate of lyophilized tumor was added to the assay tubes. Other tubes, used as controls, were heated in boiling water for 5 minutes. Following a 4-hour incubation period, the contents of each tube were transferred to narrow centrifuge tubes, and the solid matter was spun down. The supernate was discarded, and the solid matter was washed twice in 10 ml of distilled water; the washings were also discarded. The remaining solids were weighed, transferred to Ten Broeck homogenizers, and homogenized in distilled water to 15 ml total volume. Five ml of the homogenate were taken for DNA extraction by the procedure of Schmidt and Thannhauser(4); other fractions extracted by this procedure were discarded. The DNA was dried on aluminum discs, and C^{14} radioactivity was determined by employing an automatic, windowless, gas-flow counter developed in this laboratory(5). Values of phosphorus concentration of the DNA were obtained utilizing the colorimetric method of King(6). Adenine-8-C^{14} was synthesized by the procedure of Clark and Kalckar(7). The tumor tissue added to assay tubes was obtained from transplantable mammary carcinoma E-0771. The tumor was palpable in 10 days and lethal in 30. Tumors were removed approximately 20 days after subcutaneous inoculation and were homogenized in Ten Broeck homogenizers.

To confirm these results, a series of experiments was carried out on liver and also on lung minces. Normal 5- to 10-week-old BAF^1 mice were used and mammary carcinoma E-0771 obtained from BAF^1 tumor bearing mice.§ The technic used was essentially the same as described above except for the following: exactly 4 ml of Ringer's glucose in phosphate buffer (pH 7.4, ionic strength .01) was used in each tube; stainless steel planchets were used for counting on an automatic Tracerlab Superscaler; and the Adenine-8-C^{14} used was obtained from Isotopes Specialties Co. The specific activity of the preparation was 1.93 mc/mM.

Results. The results are expressed in units of disintegrations/second/gram wet weight of washed minced tissue. Values are also calculated in terms of disintegrations/sec./mg DNA phosphorus, and no significant differences between the 2 expressions were found. Duplicate values for each treatment were prepared where 2 values are given.

Groups 5, 6, and 7 were carried out under slightly different conditions and the 2 experiments should not be directly compared, but it is noteworthy that Group 5 falls within the range determined in Group 1.

That *in vitro* incorporation of adenine ac-

TABLE I. Values of Radioactivity for DNA Extracted after Incubation with Adenine-8-C^{14}.

Group	Treatment of tissue mince	Dis/sec/g wet wt of washed mince tissue
1.	Untreated	4.8, 7.5
2.	Tissue mince heated 5 min. in boiling water	.9, 1.2
3.	Tissue mince + 100-200 mg homogenized tumor tissue	8.3
4.	Tissue mince + lyophilized tumor homogenate corresponding to 400 mg tumor tissue	6.0, 8.9
5.	Untreated	5.5
6.	Tissue mince + 25 mg tumor homogenate	18.4
7.	Tissue mince + 50 mg tumor homogenate	16.2

§ C57 bl and BAF^1 mice obtained from the Roscoe B. Jackson Memorial Laboratories, Bar Harbor, Me.

TABLE II. Values of Radioactivity for DNA Extracted after Incubation with Adenine-8-C^{14}.

Group	Treatment of liver mince	Dis/sec/g wet wt of washed mince tissue
8.	Untreated	0
	Liver mince heated 5 min. in boiling water	0
	Liver mince + 25 mg homogenized tumor tissue	3.5
9.	Untreated	0
	Liver mince heated 5 min. in boiling water	0
	Liver mince + 25 mg homogenized tumor tissue (5 Mc adenine-8-C^{14})	25.4
10.	Untreated	0
	Liver mince heated 5 min. in boiling water	0
	Liver mince + 25 mg homogenized tumor tissue	3.7
11.	Untreated	0
	Liver mince heated 5 min. in boiling water	0
	Liver mince + 25 mg homogenized tumor tissue	17.6

tually does occur is indicated by the decreased radioactivity in the tubes where the mince was deactivated by heat. The stimulating effect of tumor tissue on the uptake of adenine into DNA appeared variable, but gives further evidence that active uptake mechanism was operating *in vitro*. It was found in the earlier work referred to above(2) that stimulation of adenine uptake into DNA *in vivo* was also variable. In general, the variability was thought to be due to the lability of the factor(s) in solutions of tumor homogenate.

The results of Group 4, Table I, might indicate that some of the active principle in tumor homogenate can be preserved by lyophilization. The results of Groups 5 and 7 suggest that there may be a limit to the capacity to stimulate adenine uptake. In addition, they indicate that the increased radioactivity subsequent to the addition of tumor homogenate is not merely the effect of the added "active" DNA of the tumor cells, since, in that case, Group 7 should be much higher in value than Group 6.

It was not felt necessary to maintain strictly sterile conditions during the preparation and incubation of the tissue mince since the short incubation time was not adequate for extensive growth of microorganisms. Microbiological destruction or incorporation of adenine-8-C^{14} would have had to have varied from tube to tube to have seriously affected the results.

The results shown on Table II indicate that the same increase in the uptake of labeled adenine is obtained when tumor homogenate is added to a liver mince. This was verified in 4 studies. In no instance was this effect noted in lung minces. A final control study was carried out to ascertain the amount of uptake of adenine by tumor tissue. A tumor mince was prepared under the same conditions as the untreated and the heated controls as described above. The uptake by 25 mg of tumor mince was calculated and found to be 0.5 d/sec. while the heated tumor mince showed no uptake. Thus, the uptake by the amount of tumor added to the liver mince is only a small percentage of the total obtained for the liver plus the tumor, and we may assume that the radioactivity recorded represents uptake by the liver DNA fraction.

Summary. C57 bl mouse lung-liver mince and BAF[1] mouse liver mince incubated with adenine-8-C^{14} showed greater DNA activity than a similar system which had been deactivated by heat. This increased activity was not found with lung mince. A factor(s) present in tumor homogenate and possibly also in lyophilized tumor homogenate increased the DNA radioactivity to values significantly higher than those from tissues incubated without tumor homogenates.

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