menting the deficient maternal diet with vit. B_{12}.

Conclusions. Female rats deprived of B_{12}, from before the time of mating until the end of gestation, produced deficient and weak progeny. This was shown in weights well below normal and arrested development proportionate to the subnormal weights. The defects involved vascular blocks especially in the liver with consequent passive congestion. The heart and kidneys were immature. Proximal convoluted tubules became blocked and transformed into distended tubules histologically like distal tubules. Spleens showed a loss of primitive reticular cells and transformation into large densely chromatic and pyknotic cells. The origin of the fat by which the liver sinusoids were blocked is not known.

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Accumulation of Homologous Radioiodinated Albumin in Experimental Tumors. (21963)

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Heterologous proteins and amino acids have been shown to localize selectively in transplantable and spontaneous tumors of animals(1,2). This study was undertaken to determine whether homologous proteins are concentrated in tumor tissue since homologous albumin (human serum albumin) tagged with radioactive iodine is used extensively as a diagnostic tracer agent for the detection of brain tumors and metastatic lesions of the liver(3).

Several attempts to measure the distribution of radioactivity in biopsy specimens from tumors in humans have been inconclusive because of low activity in the tissues when permissible tracer doses were used. Accordingly, it was decided to study the problem in animals inoculated with experimental tumors, using homologous albumin.

Method. Rabbit albumin was obtained by ammonium sulfate separation, dialyzed and immediately lyophilized. Electrophoretic patterns indicated the material to be albumin, the single characteristic peak being visible. The albumin was trace iodinated with I^{131} according to the procedure of Gleason and Tabern (4), and yielded a protein of high specific activity (100-600 μc/ml with an albumin content of approximately 10 mg/ml). Ultracentrifugation and electrophoretic analysis have

TABLE I. Ratio of Radioactivity of Tumor and Invasive Zone to Normal Liver.

Rabbit No.			Day sacrificed	Counts/sec./g Normal liver	Counts/sec./g Invasive zone	Counts/sec./g Tumor	Invasive zone / Normal liver	Tumor / Normal liver
4	No Lugol's	Unperfused	1	5884	8461	12373	1.44	2.10
12	Lugol's	"	1	2938	4891	3661	1.66	1.25
6	No Lugol's	"	2	3191	4589	5535	1.44	1.73
26	Lugol's	"	3	3347	3174	9436	.94	2.82
16	"	"	5	474	618	720	1.30	1.52
						Avg	1.36	1.88
34	Lugol's	Perfused	1	711	894	1398	1.26	1.97
35	"	"	2	337	512	1816	1.52	5.39
30	"	"	3	680	748	2524	1.10	3.72
31	"	"	4	453	494	1745	1.09	3.85
						Avg	1.24	3.73

shown the end product to be similar to native albumin. The tagged albumin was injected intravenously into 9 albino and Dutch rabbits with actively growing Brown-Pearce tumors of the liver. The suspension of Brown-Pearce tumor was obtained by straining a thick homogenate through 2 layers of surgical gauze. Under ether anesthesia a laparotomy was done and about 0.2 ml of the tumor cell suspension was injected into the liver under direct vision. The rabbits were examined daily and after the tumor transplants had become palpable (5-23 days following inoculation), the iodinated albumin was injected intravenously in volumes of 1-5 ml. Some of the animals were given Lugol's solution in their drinking water in order to block their thyroid glands and prevent uptake of inorganic I^{131} from the injected albumin. The animals were divided into 2 groups: the animals of the first group were sacrificed without perfusion 1 to 5 days following the injection of the labelled albumin. They were killed by ether inhalation and autopsied immediately. The other animals were perfused 1 to 5 days following the injection of labelled albumin as follows: under ether anesthesia heparin was injected intraveounsly and a cannula was inserted into the right external jugular vein. Normal saline was injected into this cannula. Blood was drained through a cannula in the left common carotid artery until only saline was obtained. As soon as that happened, the thorax was opened and normal saline was injected into the descending aorta and drained through the inferior vena cava so as to perfuse the abdominal viscera. Tissue samples were then removed. Tumor tissue was carefully separated from normal liver tissue and sections through the "invasive" zone were taken. This "invasive" zone consisted of an area at the periphery of the tumor with equal amounts of tumor and normal liver tissue to each side of the center of the section. This was checked by histologic examination. The tissue samples were minced, blotted on filter paper and weighed into culture tubes. They were then dissolved in 2 ml 2N NaOH in a boiling water bath and, after they had cooled, were counted in a sodium iodide (thallium activated) scintillation well counter. The counts recorded were corrected for physical decay to the time of the administration of the dose.

Results. Table I shows the radioactivity expressed as counts/second/gram for normal liver, tumor and "invasive" zone tissue in Brown-Pearce inoculated animals. It can be seen that consistently higher activity is present in tumor tissue than in normal liver tissue. In the perfused animals, this difference appears to be somewhat greater.

Expressed as tumor/normal liver the ratios are 1.25 and 2.82 (average 1.88) in the unperfused animals and 1.97 to 5.39 (average 3.73) in the perfused animals. The ratio of "invasive" zone/normal liver in the unperfused animals ranged from 0.94 to 1.66 (average 1.36): in the perfused animals, it was 1.09 to 1.52 (average 1.24).

Scintigrams(5) showed concentration of radioactive iodine in the liver. There was not enough activity in the tumor, however, to ob-

tain satisfactory differentiation between normal liver and tumor with this method. This is evident from the ratios of tumor tissue/normal liver tissue.

The administration of Lugol's solution to some of the animals did not change the concentration of the iodinated albumin or the ratios between tumor, "invasive" zone and normal liver tissue (Table I).

Comment. Duran-Reynals(1) showed that heterologous proteins and dyes injected intravenously localize selectively in transplantable and spontaneous tumors of mice. He interpreted his findings as, "indicating that newly formed capillaries of tumors are, in general, more permeable than the capillaries of any normal tissue." In our animals, increased concentrations of homologous albumin were demonstrated in tumor tissue as compared to the surrounding normal liver. The ratio of specific activity of tumor/normal liver was even greater after the animals had been perfused. The reason for this may, indeed, be increased permeability of newly formed capillaries with escape of homologous albumin into tumor tissue. Once the albumin has escaped into tumor tissue, it cannot be removed by perfusion. Another possibility is actual metabolic utilization of homologous albumin by the more rapidly growing tumor tissue.

The labelled protein used in our study was homologous and presumably not denatured. It is assumed that it is handled by the rabbit like native albumin. Whether the intact protein molecule or a metabolite of this protein is deposited in tumor tissue cannot be stated at this time.

Conclusion. The data presented indicate that experimental tumors of the liver (Brown-Pearce) in rabbits accumulate more radioiodinated rabbit albumin than normal liver tissue of the same animal. This increased concentration appears to be unrelated to vascularity since it was demonstrated in perfused animals.

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Fecal Androgen, A Progesterone Metabolite in the Bovine.* (21964)

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Early investigations clearly indicated that pregnanediol is a product of metabolism of progesterone in man(1-4). Further *in vivo* studies have indicated that progesterone may be reduced to, and excreted as, allopregnanediol-3(α), 20(α)(5) and pregnanol-3(α)-one-20(6). However these metabolites in the urine account for only 5-20% of administered progesterone(7). Recent studies indicate that pregnanediol is not present in urine of pregnant goats(8) and cattle(9). The importance of a biliary pathway for elimination of progesterone metabolites has been indicated: (a), recovery of pregnanediol from bile of humans after progesterone was given parenterally (10); (b), identification of allopregnanediol-3(β), 20(β) in cattle bile(11); and (c), isolation of pregnanol-3(α)one-20 and pregnanediol-3(α), 20 (β) from bile of pregnant cows (12). It has been shown in studies of proges-

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