mg/100 g blood/hour, with pyridoxine 20.5 mg, so that it can be said definitely that pyridoxine under these experimental conditions has no effect on rate of alcohol metabolism.

By plotting degree of intoxication against blood alcohol concentration for each experiment, it was possible by interpolation to estimate degree of intoxication at successive decrements of blood alcohol concentration of 20 mg/100 g. By averaging these values for 7 control runs and 10 runs with pyridoxine it was possible to plot curves of degree of intoxication at different blood alcohol concentrations with and without pyridoxine. From this graph it was apparent that at no blood alcohol concentration was there a difference of more than half a degree of intoxication that could be ascribed to pyridoxine, and where a difference occurred, this was most often a greater degree of intoxication with pyridoxine than without.

Conclusion. In the dog pyridoxine is ineffective in either increasing rate of metabolism of alcohol or reducing degree of intoxication at a given blood alcohol concentration. This is confirmatory of previous work in man, and is conclusive evidence of the uselessness of pyridoxine as an antagonist of alcoholic intoxication.

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Localization of I^{131} Labeled Antibody to Rat Fibrin in Transplantable Rat Lymphosarcoma.* (24592)

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It was possible to prepare an antibody obtained from antisera to the transplantable Murphy-Sturm rat lymphosarcoma that, after labeling with I^{131} and intravenous injection into tumor bearing rats, will localize with a high degree of preferentiality in this tumor (1,2,3). Our recent work indicated that such labeled antibody prepared against this tumor grown in rats, showed a very low degree of tumor localization when injected intravenously into hamsters bearing this tumor as a cheek pouch transplant(3). Also *in vitro* tests indicated that the hamster-grown tumor showed a weak immunological reaction with antibody prepared against the rat-grown tumor. These results combine to suggest that an important antigen in the rat-grown tumor responsible for *in vivo* antibody localization might be some substance produced or deposited by the rat host rather than some antigen intrinsic to the tumor cell itself. Day, Pressman, and associates have recently reported (personal communication) and we have confirmed that *in vivo* localizing antibody isolated from Murphy tumor antisera shows a strong tendency to react immunologically with the rat fibrinogen-fibrin system(4). This suggests that an important antigen responsible for localization of antibody in the rat Murphy tumor might be fibrinogen or fibrin deposited in the tumor by the host, possibly in response to an inflammatory reaction by host tissues to tumor growth. We have therefore immunized rabbits with rat fibrin, clotted the rabbit antisera with normal rat plasma, and from the washed clot isolated and labeled with I^{131} a substance, presumably an antibody, that shows a strong preferential tendency to localize in the Murphy-Sturm lymphosarcoma.

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Methods. Rabbits were immunized against rat fibrin by intramuscular injections of this antigen together with the complete Freund adjuvant once a week for 4 weeks, and pooled antiserum obtained by combining sera from bleedings 10 and 14 days after the last immunizing injection. To a 20 ml portion of such antisera were added 10 ml of pH 8 borate buffer, 16 ml of a saline solution of thrombin containing 40 units/ml, and 8 ml of 2% calcium chloride. The calcium chloride was slightly in excess of that necessary to give free calcium ions after addition of citrated rat plasma. Auxiliary experiments indicate quantities of borate buffer and thrombin are probably not critical. Excess thrombin was added to induce rapid clotting, primarily because of strong inhibition exerted on clotting of rat plasma by this anti-rat fibrin rabbit serum under conditions where rat plasma was not in large excess. Forty ml of rat plasma, containing 16 ml of 2% sodium citrate, were stirred rapidly into the above mixture. A definite but weak clot occurred in about 3 minutes. After 15 minutes at room temperature it was centrifuged 15 minutes at 13,000 rpm in Servall refrigerated angle centrifuge, and the compact fibrin clots homogenized in a mixture of 10 ml of a 3.2% solution of sodium salt of polyglutamic acid and 20 ml borate buffer. The high molecular weight polyglutamic acid was furnished us through the courtesy of Dr. Elkan R. Blout(5) and before use as a reagent in these experiments reacted with free I^{127} iodine to remove sites potentially competing with antibody for I^{131} during subsequent labeling procedures. Other reagents and general procedures have been described(6). The washed fibrin residue obtained by centrifuging and discarding the supernatant fluid was then mixed with 7 ml of 32% urea in polyglutamic acid plus borate buffer to elute into solution antibody to fibrin or fibrinogen present in the washed fibrin residue. Urea has the property of breaking bonds between insoluble antigen and antibody without permanently denaturing antibody(7). After stirring 5 minutes at room temperature and centrifuging at 0°C, the clear supernatant was dialyzed overnight at 0°C against borate buffer to remove urea. The 6.3 ml of dialyzed preparation contained 0.024 mg tyrosine/ml as determined by Lowry *et al.* method modified by omission of the copper reagent(8). This is assumed to correspond to 0.4 mg protein/ml exclusive of polyglutamic acid which gives a zero blank. After storing frozen for a few days a 2 ml portion of this preparation was labeled with I^{131} after preliminary purification by CCl_4 extraction of the radioactive iodine and use of a jet method for mixing on addition of I^{131} as free iodine to protein(6). About 2.6 millicuries of I^{131} were firmly bound to protein; this represented 26% of the starting I^{131}. On the assumption of uniform labeling and of an antibody molecular weight of 160,000, six atoms total iodine were attached to each molecule of antibody. After I^{131} labeling an additional purification separated labeled antibody from I^{131} containing nonspecific material. To the labeled material were added borate buffer, thrombin, and calcium chloride in proportions similar to preiodination purification to a volume of 30 ml. On addition of 10 ml of rat plasma a fibrin clot formed, and the labeled antibody was eluted from the washed fibrin with 32% urea in normal rabbit serum plus borate buffer. Yield of this postiodination purification step was 4%, or 100 μc of I^{131} in the final purified antibody preparation. Labeled antibody from this preparation and 2 similar preparations was injected intravenously into 11 white female rats of Rochester or Carworth Farms strain, both derived from original Wistar stock. Six days before labeled antibody injection these rats had been inoculated by trocar with Murphy-Sturm lymphosarcoma. The rats were sacrificed by saline perfusion about 20 hours after the labeled antibody injection, and the radioactivity of blood, tumor, and other tissues measured using procedures described earlier(6). In other experiments distribution of I^{131} labeled normal gamma globulin from nonimmunized normal rabbits was determined in Murphy tumor bearing animals.

Results. The preparations labeled and purified in this manner, presumably antibody to rat fibrin, show a strong tendency to bind to a rat fibrin clot formed in their presence. When a small amount of such a labeled preparation is added to citrated rat plasma diluted

TABLE I. Distribution of I^{131} in Rats Bearing the Murphy-Sturm Lymphosarcoma the Day Following Intravenous Injection of I^{131} Labeled Antibody Prepared Using Rat Fibrin as Antigen, and of a Control Group Injected Instead with I^{131} Labeled Normal Rabbit Gamma Globulin.

	% dose I^{131}/g tissue	
Organ or tissue	Antibody to rat fibrin, avg values for 11 rats	Normal rabbit γ-globulin, avg values for 15 rats
Blood	3.85 ± .51	4.67 ± .86
Heart	.18 ± .06	.32 ± .07
Lung	.49 ± .18	.77 ± .55
Liver	.27 ± .06	.13 ± .06
Spleen	.55 ± .11	.45 ± .24
Kidney	.22 ± .10	.09 ± .04
Adrenal	.45 ± .18	.26 ± .14
Ovary	.40 ± .12	.70 ± .24
Lymphatic tissue	.30 ± .07	.32 ± .12
Muscle	.07 ± .04	.09 ± .04
Skin	.26 ± .11	.52 ± .18
Small intestine	.19 ± .06	.18 ± .05
Tumor	14.30 ±3.70	2.16 ± .50

These rats were sacrificed by saline perfusion approximately 20 hr after intrav. inj. of I^{131} labeled antibody or of γ-globulin. Data normalized to 100 g rats. Variability is expressed as stand. dev. of individual animal values.

by a factor of 2 with saline and clotting induced with $CaCl_2$ or thrombin, some 60-80% of the I^{131} label is found in the centrifuged and washed clot. In similar experiments with rabbit plasma only 4-8% of the I^{131} label was carried down in the clot.

The first data column of Table I shows distribution of I^{131} in tumor bearing rats injected with labeled antibody prepared with rat fibrin as antigen. Distribution of I^{131} seemed strain independent in these experiments. The second column shows distribution of I^{131} labeled normal rabbit gamma globulin *in vivo* filtered by the method of McFarlane (9) and Gordon(10). Control values for I^{131} content of tumor obtained by this procedure are higher, measured in percentage of injected dose than after injection of I^{131} labeled untreated normal gamma globulin, and thus give a more conservative estimate of I^{131} accumulation in tumors of rats injected with labeled antibody that may be nonspecific in nature (2). The rats injected with antibody to fibrin averaged 131 g in weight, and tumor weights averaged 1.31 g, or 1% of the rat weight. They contained an average of 14.3% of the injected I^{131} dose. Data on one rat measured by external gamma counting for 3 days after antibody injection indicated I^{131} content of the tumor remained approximately constant while that of blood and viscera decreased so that ratio of radiation dosage to tumor compared with normal body tissues increased with time.

Discussion. The data presented here indicate that an I^{131} labeled antibody to rat fibrin, or to some blood component associated with fibrin during the clotting of plasma, also shows *in vivo* a strong tendency to localize preferentially in the transplantable Murphy-Sturm rat lymphosarcoma. Radioactivity localization in tumor is preferential enough to suggest the possibility of effective radiation therapy of this tumor by this procedure. A possible explanation of antibody localization is that tumor growth induces an inflammatory reaction in the host leading to deposition of fibrin, and in our experiments, subsequent deposition of previously circulating antibody to fibrin. It is also possible that I^{131} labeled antibody combines with circulating fibrinogen, and with fibrinogen conversion and deposition as fibrin the antibody is at the same time deposited in tumor. It appears worthwhile to investigate whether antibody to fibrin will localize in other transplantable or spontaneous tumors. It may turn out that, even in tumors that do not normally induce an inflammatory reaction in the host, preliminary chemotherapy, or radiation therapy not sufficient in itself to destroy the tumor, can induce an inflammatory reaction that can lead to effective radioactivity localization for therapy.

Summary. Rabbits were immunized with rat fibrin and the resulting antisera clotted with normal rat plasma. From the clot a substance was isolated and labeled with I^{131} that showed a strong tendency to bind to rat fibrin clots formed in its presence. This substance, presumably an antibody to rat fibrin, when injected intravenously into rats bearing the transplantable Murphy-Sturm lymphosarcoma localized with a high degree of preferentiality in this tumor.

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Role of Liver and Kidney in Development of Heparin-induced Lipemia Clearing Activity (LCA).*† (24593)

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Since the discovery that injection of heparin endows the plasma with a lipemia clearing activity (LCA)(1,2), it has been an interesting problem to find out which tissues are essential to development or inactivation of this lipolytic enzyme system. Isolated perfusion experiments showed that heparinised plasma develops LCA after it is passed through limbs, thoracic and most abdominal viscera(3-6) but not the brain(5) or the liver(6). Indeed, when plasma already endowed with LCA was perfused through the latter organ is suffered a reduction of that activity(6,9), indicating that the liver inactivates LCA. The present study was undertaken to ascertain the effects of hepatectomy or CCl_4-induced liver damage and of nephrectomy on *in vivo* development of LCA following heparin injection in rats.

Materials and methods. The same experimental plan as in a previous study(7) was employed. Heparin (Connaught's) was injected into the tail veins of control and experimental male Wistar rats weighing 220-280 g, at 0.5 mg/kg. Five minutes after heparin injection 4.5 ml of blood was withdrawn (under ether) from the abdominal aorta of every animal, mixed 1:10 (v/v) with 0.1 molar sodium citrate and centrifuged for 15′ at 3000 rpm. In each instance, 1 ml of the "postheparin" plasma thus obtained was diluted with 1 ml distilled water and mixed in a colorimeter cuvette with 0.5 ml lipemic plasma, produced as previously described(8); turbidity of the postheparin-lipemic plasma mixture was determined immediately and 10′ and 20′ after mixing in a Klett-Summerson colorimeter, against a water blank, using a red filter. Loss of turbidity ("clearing") of the mixtures at any given time was expressed as percentage of initial turbidity at mixing. The animals were injected with heparin and their plasmata obtained and tested for LCA in successive pairs consisting of one control and one experimental, following a precisely timed, staggered schedule. Since the same lipemic plasma batch was used as clearing substrate for each pair of control and experimental plasmata, the results were analyzed statistically with the Null Hypothesis(12). Thus, in every experiment, the mean difference $\pm \epsilon$ between the clearing of every control and its corresponding experimental mixture was computed for each time interval. In tabulating these differences the clearing activities exhibited by the control plasmata were arbitrarily set as references against which those of the experimental plasmata were compared. A difference with a

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