affect the tissue, but eventually do so, leaving no reservoir of steroid in the liquid phase. To test the validity of this conclusion, the preparations resulting from 60-minute incubation of the brain preparation with 1, 2, and 4 mg of DC at 37.2°C were filtered and the filtrates examined for steroid by Zimmerman reaction and for effect on respiration of other brain suspensions. No steroid could be detected in the filtrate by either method. The amount of DC necessary to produce minimal significant inhition of aerobic respiration *in vitro* was 60 μg, a greater concentration than that which has been shown to affect other systems. For example, Bartlett and Mackay(7) have found that 30 μg of DC reduce the amount of glycogen formed when skeletal muscle is incubated with glucose.

*Summary.* (1) The concentration-action relationships of DC and DCG upon aerobic respiration of rat brain cell suspensions indicate:

(a) Increasing amounts of DC and DCG produce increasing amounts of inhibition This relationship is reasonably linear plotting the logarithm of the amount of DC against per cent of inhibition produced.

(b) With increasing amounts of DC a longer period of time is required to attain maximal inhibition while a comparable effect is more rapidly attained with equimolar amounts of the water-soluble conjugate, DCG. The effect, once attained, remains constant without further increase or release of the inhibition.

(c) By this bio-assay the water-solubility of DC at 37.2°C is approximately $3.6 \times 10^{-3}$ Molar.

(2) These data suggest that, under the conditions used, the relatively large amounts of steroid necessary to produce inhibition of aerobic respiration *in vitro* entirely penetrate or are adsorbed by the affected system. These amounts exceed the concentrations of steroid which will affect other systems *in vitro* or that occur in living organisms. This does not necessarily exclude the possibility that steroidal inhibition of aerobic respiration may be of significance in the intact organism.

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## Specific Localization of Anti-Rat-Lung Serum in the Lung.* (17606)

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Up to the present time, the only direct evidence that antibodies prepared against an animal's tissue actually localize in the homologous tissue has been the demonstration, with the use of radioactive tracers, that antibodies prepared in rabbits against rat kidney or mouse kidney do localize in the kidneys of rats or mice.(1-5) In order to observe whether tissues other than kidney can concentrate antibodies from tissue-homologous antisera, or whether kidney is unique in this property, we have investigated antisera against several other rat tissues.

We now have evidence, which we are reporting here, that antibodies prepared against rat lung localize in the lung. Experiments were also carried out with antisera pre-

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pared against rat brain, liver, and heart and with a control non-tissue antiserum, namely, antiovalbumin. No specific localization was observed with these anti-tissue sera as compared to the antiovalbumin control. However, we have reason to believe, as discussed presently, that antisera prepared differently may yield positive localization in some of these tissues, also.

The anti-rat-tissue sera were prepared in rabbits by the injection of antigens obtained by homogenizing the perfused tissue(2) of rats of the S. C. Wistar strain supplied by Dr. Deuel of the University of Southern California. The anti-rat-kidney serum and the antiovalbumin serum were the preparations described previously.(2) For the production of anti-rat-lung, -brain, and -heart sera, two rabbits, in the case of each tissue, were injected intraäbdominally twice a week on consecutive days for 3 weeks with 5% suspensions of brain or lung or a 2½% suspension of heart. The animals were bled from the ear one week after the last injection. A subsequent bleeding was obtained after a second course of 2 weeks of 2 injections per week of 2 ml of a 15% suspension of the tissue. The sera of the 2 bleedings for the 2 rabbits were pooled since they all showed similar titers in precipitation reactions. The anti-liver serum was prepared by injecting 2 rabbits intraäbdominally twice a week on consecutive days for 2 weeks with a 30% suspension of liver tissue and bleeding the animals one week after the last injection. The globulin fractions of these sera were prepared by the alcohol fractionation method described previously.(3) All of the sera and the globulin fractions thus prepared showed precipitation when they were titered with the homologous antigen. There was appreciable cross reaction with other anti-tissue sera, but no precipitate was obtained with these antigens and normal serum. The antigens for this test were prepared by centrifuging a 10% suspension of the perfused tissue for 30 minutes at 1000 RCF and using the somewhat cloudy supernate. Both S. C. Wistar and Sherman albino rats were used to supply the test antigen preparations.

The globulin fractions of the antisera were iodinated with iodine containing tracer amounts of the radioactive $I^{131}$ as described previously.‡(4)

Each of the radioiodinated globuin fractions of the antisera was injected intravenously into several Sherman albino rats of about 150 g weight. About 0.7 mg of protein and 10 $\mu$c ($1.4 \times 10^6$ counts per min.) of radio-activity in 0.5 ml of solution was used for each injection. At intervals of 3, 6, 10, 13, 18, and 25 days after injection, one animal in each group was sacrificed, perfused, and the tissues analyzed for radioactivity as described elsewhere.(3)

The results are shown in Table I where the concentrations of radioactivity (counts per minute per gram) of blood or of wet perfused tissue, *i.e.*, lung, kidney, liver, brain, heart, and spleen on the various days after injection are tabulated for the different antisera used.

In the case of the lung, a much higher concentration of radioactivity localized from the anti-lung serum than from any of the other sera used. Indeed, none of the other anti-tissue sera were significantly more active in this respect than the antiovalbumin control. In the case of the kidney, there was high concentration of radioactivity localized from the anti-kidney serum as was described previously(2) and an essentially equivalent high concentration of radioactivity from the anti-lung serum. The kidney-localizing antibody in the anti-lung serum appeared to be quite similar, with respect to extent and duration of localization, to the kidney-localizing antibody in the anti-kidney serum. The extents of localization of the anti-lung serum in the kidney and in the lung were essentially the same initially, but the duration was much less in the lung than in the kidney so that the relative concentration in the kidney became greater with time. The anti-brain and anti-heart sera seemed to show very slightly greater localization in kidney than did the anti-liver serum and the antiovalbumin serum control. This greater localization may have been real but it was still much less than that observed with anti-kidney and anti-lung sera.

‡ Carrier-free radioiodine was obtained from the U. S. Atomic Energy Commission, Oak Ridge Operations, Isotopes Division, Oak Ridge, Tenn.

TABLE I.

Localization of Radioactivity in Various Tissues from Radioiodinated Globulin Fractions of Various Anti-tissue Sera.

Sherman albino rats were injected intravenously with about 0.7 mg protein and 10 $\mu$c ($1.4 \times 10^6$ counts per minute). The animals were perfused on the days indicated and the tissues analyzed.

| Tissue | Antiserum used | Radioactivity in tissues (Counts per g per min.)* | | | | | |
|---|---|---|---|---|---|---|---|
| | | 3rd day | 6th day | 10th day | 13th day | 18th day | 25th day |
| Lung | Anti-l† | 8650 | 2390 | 1000 | 320 | 230 | (44) |
| | " k | 2400 | 1280 | 320 | | | (21) |
| | " li | 2230 | 270 | (85) | (40) | (13) | ( 0) |
| | " b | | 380 | 230 | | | |
| | " h | 1350 | 630 | 310 | | | |
| | " o | 1880 | 770 | (60) | (20) | (13) | ( 8) |
| Kidney | Anti-l | 9380 | 5900 | 4180 | 2670 | 1480 | 770 |
| | k | 11200 | 6700 | 7050 | | | 920 |
| | " li | 1980 | 550 | 380 | 350 | 230 | (30) |
| | " b | 2760 | 1760 | 1020 | | | |
| | " h | 1150 | 2140 | 1200 | | | |
| | " o | 680 | 390 | 650 | 870 | 290 | (42) |
| Liver | Anti-l | 3340 | 1540 | 580 | 230 | 300 | 100 |
| | " k | 2170 | 970 | 640 | | | 50 |
| | " li | 3340 | 570 | 130 | 64 | 40 | ( 0) |
| | " b | 3400 | 820 | 270 | | | |
| | " h | 1180 | 930 | 380 | | | |
| | " o | 1280 | 440 | 190 | 42 | (13) | (12)‡ |
| Brain | Anti-l | 160 | (65) | (26) | | | |
| | " k | 90 | 70 | (25) | | | |
| | " li | 100 | (60) | ( 9) | | | |
| | " b | 140 | 90 | (25) | | | |
| | " h | 50‡ | 60 | ( 5) | | | |
| | " o | 70 | 90 | (12) | | | |
| Heart | Anti-l | 1120 | 570 | 110 | | | |
| | " k | 970 | 330 | 130 | | | |
| | " li | 940 | 140 | (27) | | | |
| | " b | 1170 | 280 | 150 | | | |
| | " h | 440 | 310 | 140 | | | |
| | " o | 400 | 190 | (32) | | | |
| Spleen | Anti-l | 3850 | 4850 | 1080 | | | |
| | " k | 4390 | 2420 | 1170 | | | |
| | " li | 1930 | 380 | 550 | | | |
| | " b | 3110 | 1380 | 340 | | | |
| | " h | 1990 | 1750 | 970 | | | |
| | " o | 1020 | 460 | 780 | | | |
| Blood | Anti-l | 6700 | 710 | 150 | (28) | (22) | ( 2) |
| | " k | 19700 | 3000 | 210 | | | (15) |
| | " li | 8150 | 880 | 110 | (28) | ( 9) | ( 0) |
| | " b | 8650 | 370 | 140 | | | |
| | " h | 6980 | 480 | 150 | | | |
| | " o | 14300 | 6180 | 120 | (34) | ( 6) | 64 |

* Count for wet, perfused tissues corrected to day of perfusion. Counts are averages of duplicate samples with a four-minute count and a background of about 20 cpm. Counts based upon original counts of less than 20 cpm, or less, above background are not accurate and are indicated by enclosure in parentheses.

† l, lung; k, kidney; li, liver; b, brain; h, heart; o, ovalbumin.

‡ Single sample.

In the case of the liver, previous work(5) showed that anti-mouse-kidney serum had a very low, but definite, localization with a long duration in the liver of mice. In this experiment with anti-rat-kidney serum there similarly was an indication of some localization

in liver with a long duration. With anti-lung serum also, there seemed to be definite localization in the liver with a long duration. When compared with the antiovalbumin control, there was a suggestion of liver localization for anti-brain and anti-heart sera. On the other hand, the tissue-homologous serum, the anti-liver serum, showed essentially the least localization in liver. This may have been due to the fact that the rabbits producing the antiserum had received fewer injections of antigen over a shorter time, although actually good precipitins against liver tissue were present in the serum. In the case of the brain and heart there was no significantly greater localization from any of the sera, including the tissue-homologous serum, than from the antiovalbumin control.

The amounts of localization in the spleen were very variable, although there seems to have been somewhat greater localization initially from the anti-lung, -kidney, -brain, and -heart sera than from the anti-liver serum and the antiovalbumin serum control.

The amount of radioactivity in the blood indicated the relative amounts of radioiodinated protein present in the blood on the day of analysis, and was a function of the amount of radioactivity injected and the rate of removal of the radioactivity from the bloodstream. During the first few days after injection, the radioactivity in the blood may be taken as an indication of the amount of radioactivity actually injected. Therefore, it appears that the animals receiving anti-kidney serum and antiovalbumin serum may have received somewhat higher quantities of radioactivity than the others. Hence, the relative initial localization in the various tissues from these sera was somewhat less, on a comparative basis, than the counts would indicate. The preceding discussion of the results would not be altered by any such correction.

The failure to demonstrate tissue-specific localizing antibodies in the anti-liver, -brain, and -heart sera is not conclusive. This is emphasized by the fact that we know that there are substances in liver which bind antibodies prepared against kidney and lung, indicating that there must be common or at least cross-reacting antigens in these tissues. However, the antiserum prepared against liver not only did not react with the kidney or lung where cross-reacting antigens are known to be present, but also did not localize in liver as anti-kidney or anti-lung sera did.

These data show that the anti-lung serum localized in lung. The anti-lung serum also contained antibodies which cross-reacted with substances in the liver and kidney. Although the localization in the kidney from anti-lung serum was quite similar in extent to the localization from anti-kidney serum, the localization in lung was quite different for the two sera. Thus, there must be substances in the lung which are different from those in the kidney and which are responsible for the production and localization of anti-lung antibodies. It follows from the foregoing data that kidney tissue is not unique in being able to accumulate antibodies from tissue-homologous antiserum.

*Summary.* By labelling anti-rat-lung serum with radioiodine and injecting it into rats, it was found that the anti-rat-lung serum contained antibody which localized specifically in the lung. It also contained antibody which cross-reacted with and localized in the kidney. Anti-rat-brain, -liver, and -heart sera were also investigated and showed no specific localization, but these experiments were considered inconclusive.

The data presented show that kidney tissue is not unique in being able to localize antibody from tissue homologous antiserum.

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