Autoradiographic Arsenic Localization in Adult and Embryonic Epithelium and Connective Tissues.* (20601)

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(Introduced by S. Warren.)
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It is well known that absorbed inorganic arsenic in mammals is localized in and excreted via the skin, nails and hair. However, biochemical, histochemical and isotope technics are in disagreement or leave doubt as to the exact tissue sites of predilection. As part of a pathologic study(1) which demonstrated cancers arising in human skin from epidermis, sweat and sebaceous glands, and probably also from hair follicles, an attempt was made to determine in animal tissues the localizations of radioactive arsenic, using autoradiography. Uptakes were tested in intact adult mice and in tissue cultures of embryonic mouse epithelium and chick fibroblasts.

Methods. The radioactive arsenic was received as a mixture of As^{73} and As^{74}. A single analysis showed 3.406 mg of arsenic per ml of the original solution in 0.059 N hydrochloric acid. Before injection a pH 6.4 phosphate buffer was added. The radioactivity level was redetermined before use. Mice of ABC strain, either sex, weighing 20 ± 5 g were used. Single injections were made:

Vol, ml	Buffer, ml	As^{73}, μc	As^{74}, μc	Inj. (vein)	Sacrificed
.2	.1	28.4	5.15	Jugular	1 hr
.3	.1	42.6	7.72	Caudal	20
.3	.1	42.6	7.11	"	72

There was insufficient radioactivity in mouse tissues from these experiments fixed by freezing-drying or in 10% formalin and embedded in paraffin to permit successful autoradiography.

On this account repeated doses were employed:

Vol, ml	Buffer, ml	As^{73}, μc	As^{74}, μc	Inj.	Interval
.5	.3	67.5	10.1	Intrap.	
.5	.3	67.5	10.1	"	1 hr later
.75	.2	101.25	15.0	"	22 hr after 2nd inj.

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Sacrifice was carried out one hour later. The total dose was 236.25 μc of As^{73} and 35.2 of As^{74} in 1.75 ml of arsenic solution plus 0.8 ml of phosphate buffer. Total arsenic administered was 5.95 mg.

The mouse tissues were fixed by freezing-drying and embedded in paraffin(2). Sections were cut at 6-8 μ thick, and preparations were completed on the 5th day after sacrifice. Autoradiographs set up at this time were developed after 10 weeks of exposure.

Autoradiographs demonstrated arsenic in skin sharply and strikingly localized to epidermis, hair follicles, and other skin adnexa (Fig. 1, A and B). Dermal deposit was practically nil. In other tissues such as lung and kidney localization was evident chiefly in blood vessels. In the kidney a rich juxtamedullary cortical circulation was demonstrated, suggestive of a relative ischemia of the outer renal cortex (Fig. 2, A and B). This would be consistent with incipient shock(3).

Tissue cultures were carried out by the "flying cover-slip" technic(4), using a nutrient solution composed of 4 parts of human ascitic fluid, 1 part of chicken plasma, 5 parts of balanced salt solution, and 1 part of penicillin 1000 units/ml(5). Fibroblast cultures were obtained from 8-9 day old chick embryos and embryonic mouse epithelium from C_{57} strain fetuses. Cultures were grown at 38°C.

Radioactive or ordinary arsenic trichloride was added to separate fibroblastic and epithelial cultures, in dilutions of 1 part arsenic to 80000 parts of medium. Explants cultured were a) with radioactive arsenic, b) with ordinary arsenic trichloride and c) control with ordinary media. The radioactive solution was dilute enough to permit tissue growth without morphologic evidence of radiation damage.

After 6 days incubation the cultures were fixed in 10% formalin buffered to pH 7.0 and stained with hematoxylin and eosin, or used

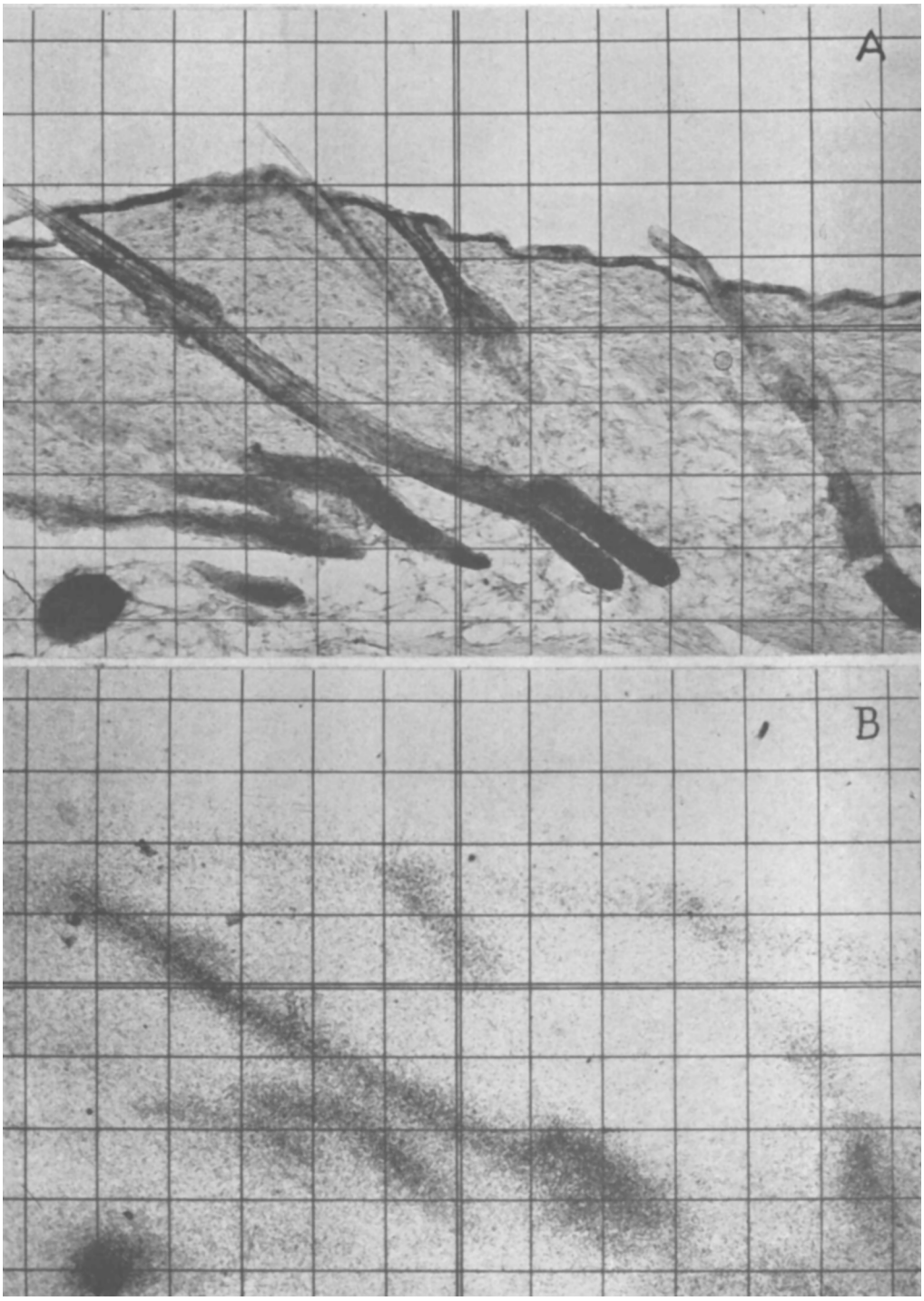

FIG. 1. a) Frozen-dried dry-mounted section of mouse skin, stained with hematoxylin and eosin after autoradiography. b) Autoradiograph from identical skin section, demonstrating arsenic radioactivity sharply localized to hair follicles, skin adnexa and epidermis. ×100.

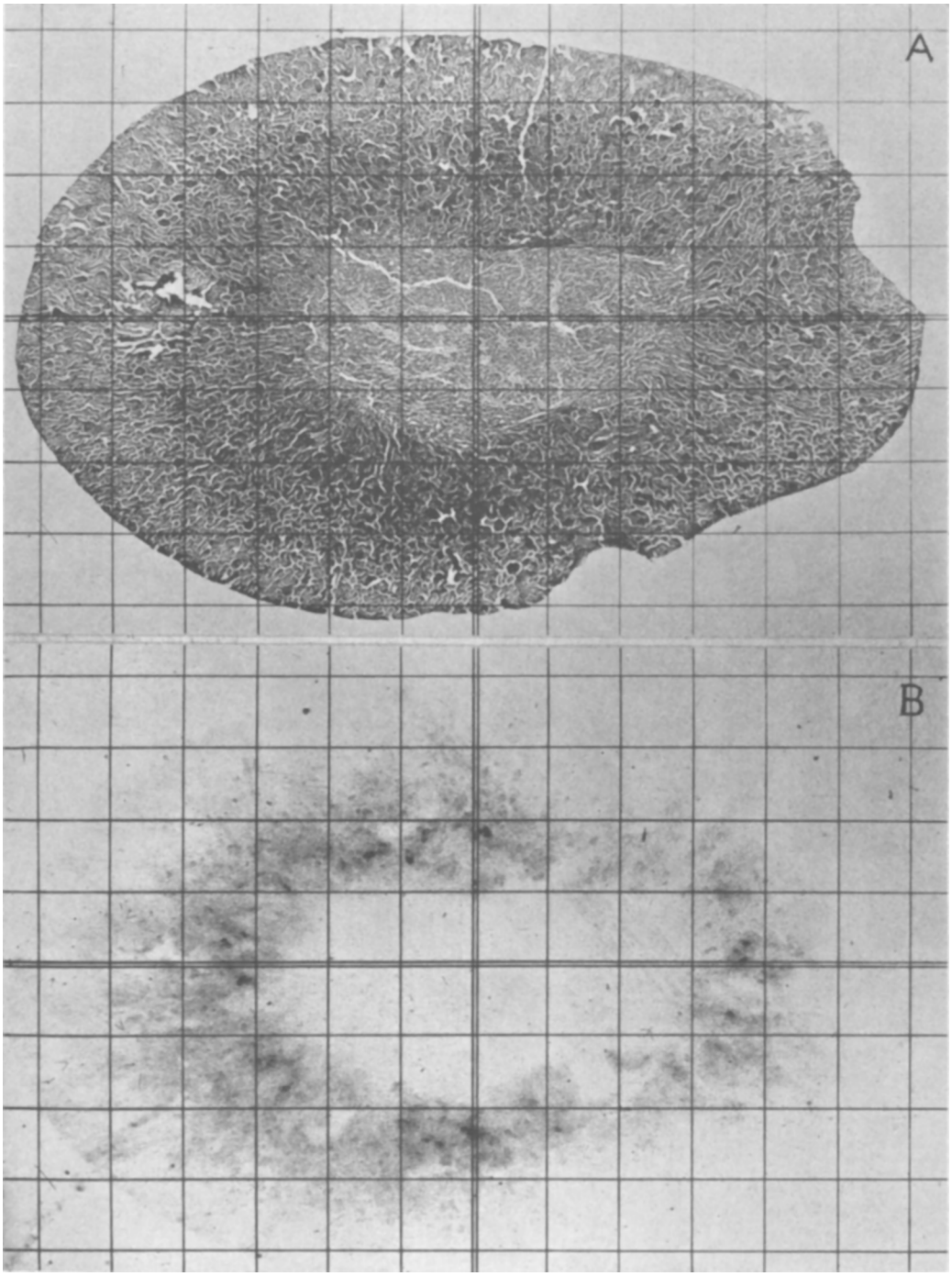

FIG. 2. a) Section of mouse kidney similarly prepared. Juxtamedullary cortical vascular congestion is present. b) Autoradiograph of identical section, showing intravascular arsenic radioactivity, following 3 injections during a 24 hr experiment. ×24.

for autoradiography, or both. Examination of both normal control cultures and those exposed to radioactive arsenic showed good histologic preservation and active proliferations at the edges composed of the respective epithelial or fibroblastic cellular components.

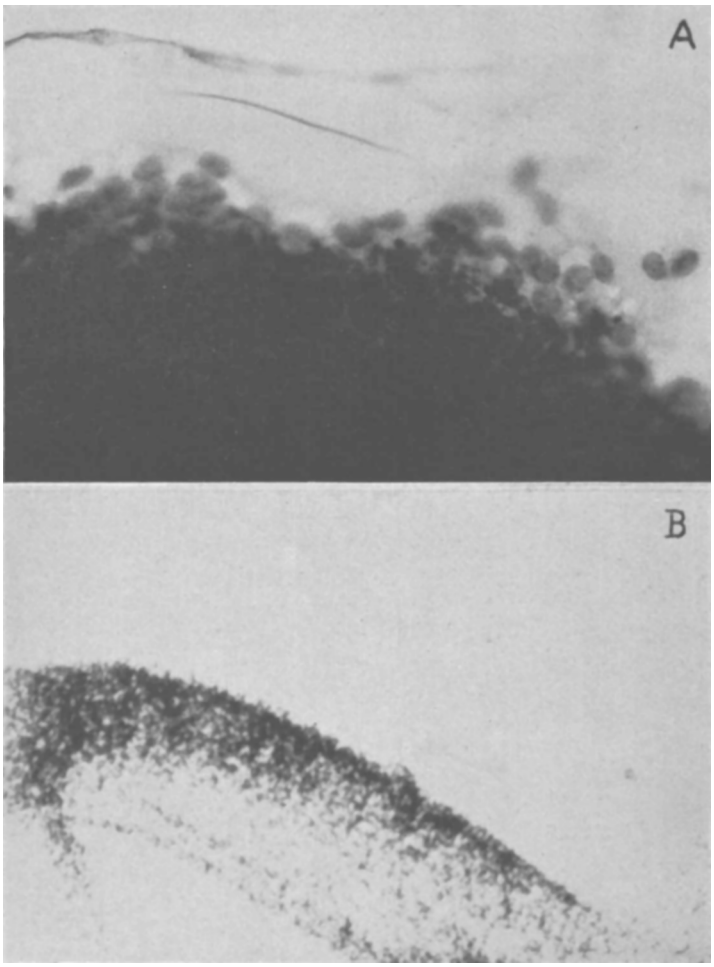

FIG. 3. Comparison of radioactive arsenic treated epithelial tissue culture margins a) stained as described, and b) as demonstrated by autoradiography. Fields are not identical. ×500.

Uptakes of radioactive arsenic were strong both in tissue cultures of embryonic epithelium (Fig. 3, A and B) and fibroblasts (Fig. 4, A and B), as demonstrated by autoradiography. The fibroblast culture uptake was slightly greater.

Controls exposed to 1:80000 dilutions of ordinary arsenic trichloride showed considerable necrosis of embryonic fibroblasts, but with some peripheral macrophage migration. Evidently the actual concentration of radioactive arsenic fell below the level of chemical toxicity. The technical difficulties of obtaining sufficient isotopic arsenic limited more extensive explorations.

Histochemical tests(6) for arsenic carried out on the same tissues were negative.

Comment. To our knowledge, use of radioactive inorganic arsenic in autoradiography has not been previously reported, although As^{74} tagged lewisite localization in skin has been studied(7). The autoradiographic results have suggested a contrast in the metabolism of arsenic in adult versus embryonic tissues. The epidermis and its adnexa were the specific sites of localization in the adult, while both embryonic fibroblasts and epithelium in tissue cultures absorbed arsenic with almost equal vigor. It would appear that the carcinogenic arsenic is actually contained within the types of cells where neoplasms later develop. Histochemical tests used at present thus fail accurately to localize the arsenic and therefore are not useful in such investigations

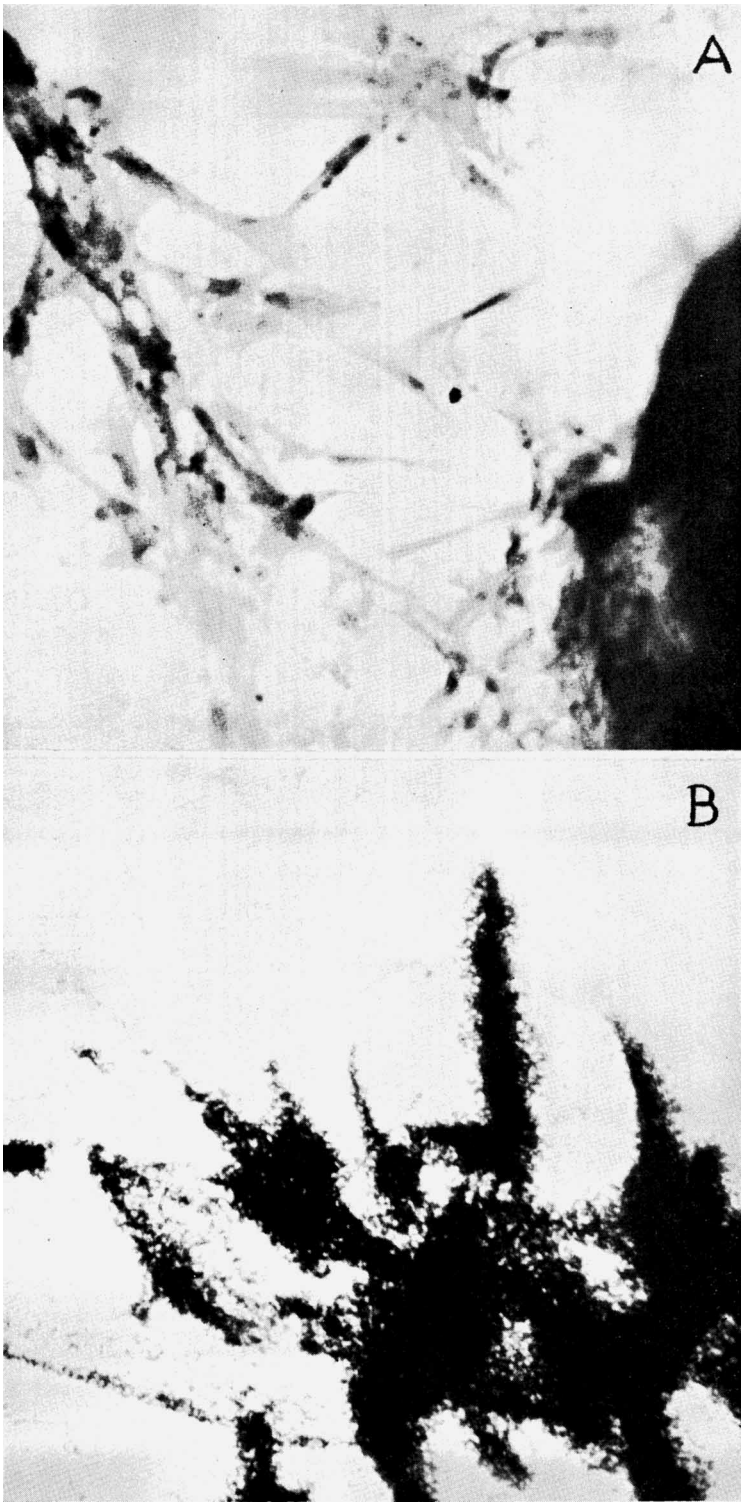

FIG. 4. Radioactive arsenic treated fibroblast tissue culture for comparison of margins a) stained as described b) shown by autoradiography. Fields are not identical. ×500.

of human tissues.

Summary. Autoradiography showed specific localization of As^{73} and As^{74} in adult mouse epidermis and skin adnexa. In tissue cultures embryonic mouse epidermis and chicken fibroblasts both absorbed the radioactive arsenic. Histochemical methods so far developed have evidently not accurately demonstrated the locations of arsenic in tissues.

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1. Sommers, S. C., and McManus, R. G., *Cancer,* 1953, v6, 347.

2. Holt, M. W., and Warren, S., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 545.

3. Trueta, J., Barclay, A. E., Daniel, P. M., Franklin, K. J., and Prichard, M. M. L., *Studies of the Renal Circulation,* 1947, C. C. Thomas, Springfield.

4. Montgomery, P. O'B. and Warren, S., *Science,* 1953, v117, 589.

5. Parker, R. C., *Methods of Tissue Culture,* 1950, P. B. Hoeber, Inc., New York.

6. Cowdry, E. V., *Laboratory Technique in Biology and Medicine,* 1948, Williams & Wilkins Co., Baltimore, 27.

7. Axelrod, D. J., and Hamilton, J. G., *Am. J. Path.,* 1947, v23, 389.

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Time-Response Effect of Cortisone upon Liver Glycogen in the Rat. (20602)

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The acute glycogenic effect of cortisone is known. In the present experiments cortisone acetate was administered to force-fed rats for periods up to 6 weeks. The peak level of liver glycogen was reached within 5 days and thereafter began to decline but remained at higher-than-normal levels during the times studied here.

Methods. Male rats of the Sprague-Dawley strain, having an initial weight of approximately 300 g, were force-fed a medium carbohydrate diet(1). All of the animals were force-fed for 14 days prior to beginning the injection of cortisone acetate. Cortisone acetate (microcrystalline suspension, Upjohn) was administered subcutaneously each morning. At the end of the injection period each rat was fasted for 24 hours and anesthetized with cyclopal; the liver was rapidly excised, weighed and dropped into hot KOH. Liver glycogen was determined by the use of anthrone(2).

Experiments and results. The cortisone dosage was 2, 5 and 10 mg/rat/day. At each of the 3 dosage levels, 6 to 8 rats were killed at intervals of 1, 2, 3, 5, 7, 10, 14, 21, 28 and 42 days. Liver glycogen was determined in 8 untreated rats. Rats given 10 mg of cortisone daily developed generalized infection between 4 and 6 weeks. Four of these rats died and the remaining 3 were sick and unsuitable for the determination of liver glycogen. The average values are summarized in Fig. 1. The increase was proportional to the dose of cortisone acetate. The peak level was reached by 5 days at each of the 3 dosage levels. Thereafter, there was a decline in the amount of liver glycogen but the time-response curves (Fig. 1) did not approach the values for untreated animals during the periods studied here. The values were also calculated as total glycogen per rat and as per cent glycogen in liver. Although these values did not correlate exactly with those shown in Fig. 1, the shape of the curves remained essentially the same. The concentration of glycogen in liver reached 9.26% for the group treated with 10 mg of cortisone acetate daily for 5 days.

Discussion. The decline in the time-response curve showing the effect of cortisone upon the level of liver glycogen may represent