Reports

Influence of Adult Tissue Homogenates on Formation of Similar Embryonic Proteins

In 1916 Murphy (*1*) discovered that chorioallantoic grafts of adult chicken spleens cause an enlargement of the spleens of host chick embryos. After an eclipse of some 20 years, these and related phenomena have become the subject of renewed interest, largely as a result of the investigations and commentary of P. Weiss (*2*). An investigation of this effect by means of tracer methods has recently been initiated by Ebert (*3*), who has shown that, as a result of the graft of labeled adult tissues, the specific radioactivity of the corresponding host organ is considerably higher than that of other embryonic tissues. We have extended these investigations to the use of injected tissue homogenates. While this paper was in preparation, there appeared a report by Tumanishvili *et al.* (*4*) that heart and liver extracts, when they are injected into chick embryos lead to specific enlargements of host organs.

In the experiments reported here (*5*), chicken-liver and heart homogenates were prepared and radioactively labeled in their proteins with S^{35}-sulfur amino acids. *Torulopsis utilis* was grown on S^{35}-sulfate (obtained from Oak Ridge National Laboratory), according to the method of Wood and Perkinson (*6*). After extraction with organic solvents (*7*), the yeast proteins were hydrolyzed with a 1/1 mixture of 6*N* HCl and 90-percent formic acid. The humin was removed by centrifugation, and a portion of the neutralized hydrolyzate corresponding to 50 mc of the original S^{35}-sulfate was injected intraperitoneally into a White Leghorn hen in two doses about 5 hours apart. Eight hours after the second injection, the blood was withdrawn by heart puncture, and the tissues were frozen.

Several months later the tissues were thawed and homogenized in a tris(hydroxymethyl)aminomethane buffer solution at *p*H 7.4 containing 0.9 percent KCl, first in a Waring Blendor and then in a Potter-Elvehjem homogenizer. The liver and heart homogenates, made up to 10 percent (weight/volume) with the same buffer solution, were then treated with cysteine at a *p*H of 8.0 to 8.5 to assure removal of all exchangeable S^{35}. After dialysis, some undissolved material was removed by low-speed centrifugation, and the relatively clear supernatant was used for intravenous injection into 9-day-old White Rock chick embryos, as described in a subsequent paragraph. Aliquots of the homogenates were mixed with carrier serum and precipitated with trichloroacetic acid; dry protein powders were prepared and counted as described by Walter *et al.* (*8*). All counts were corrected for self-absorption and decay.

Embryonated White Rock eggs were incubated at 38°C under controlled humidity conditions for a period of 9 days. They were candled on the ninth day to locate the blood vessels, and the location of the latter was marked on the shell of each egg. An area of about 1 cm^2 of the shell above the vessel was carefully cut out by means of a dental drill and burr without injuring the membrane, and the small square was removed with a razor blade. A drop of mineral oil was placed on the membrane to render it transparent, and 0.1 ml of the liver or heart homogenate was intravenously injected in the direction of blood flow. The eggs were reincubated for 24 hours and the embryos excised. Hearts and livers were removed, pooled, and homogenized; dry protein powders were prepared for counting according to the method cited in a previous paragraph.

The results of these experiments are given in Table 1. In all, two series of experiments make up the table. In the first series, 24 embryos each were injected with heart and liver homogenates; of these, 22 and 11, respectively, survived. In the second series, 44 out of 47 embryos injected with the heart preparation survived, while the number of survivors was 22 out of 28 for the liver homogenate. Thus the table summarizes data obtained on 99 survivors out of 123 embryos that were injected: 66/71 for heart; 33/52 for liver.

It can be seen that the relative specific activity of hearts is higher than that of livers when chicken heart homogenate is injected, whereas the relative specific activity of the livers is higher than that of hearts when chicken-liver homogenate is injected. It has therefore been demonstrated that injected homogenates give results qualitatively and quantitatively similar to those obtained on grafting chicken tissues to the chorioallantois (*3*). A tentative interpretation of these findings is that there occurs a preferential localization of the injected material in the tissue of origin, perhaps due to the transfer of specific protein moieties. Preliminary surveys indicate that the relative proportions of sulfur-containing amino acids in the two tissues under investigation cannot account for the results observed.

We are extending these investigations to the use of subcellular tissue fractions and to heterologous (rabbit) hearts and livers in an effort to delineate the nature of the mechanism responsible for these results.

Table 1. Results of 24-hour incubation of 9-day-old chick embryos after injection with tissue homogenates.

Item	Injection							
	Chicken heart homogenate				Chicken liver homogenate			
Count/min per embryo injected	398		398		2780		2780	
Mg injected per egg	0.1		0.1		0.1		0.1	
Organs investigated	Hearts	Livers	Hearts	Livers	Livers	Hearts	Livers	Hearts
No. of organs cut out	22	11	22	11	11	11	11	11
Dry protein wt. of organs obtained (mg)	38.2	72.0	38.8	70.0	84.7	20.9	77.6	22.4
Wt. counted (mg)	18.3	29.8	23.4	30.0	30.1	11.6	30.2	12.6
Count/min observed*	21	24	22	19	366	173	389	214
Corrected count/min per 30 mg	28	24	25	19	365	286	386	340
Relative specific activity	1.00	0.86	1.00	0.76	1.00	0.78	1.00	0.87

* Counts per minute are within 5 percent standard deviation.

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Uronic Acid of Chondroitin Sulfate B

Chondroitin sulfate B has been isolated as the major mucopolysaccharide component of skin, ligamentum nuchae, tendon, and heart valves, and it has also been found in other tissues (*1*). It was differentiated from the chondroitin sulfates A and C by its rotation, its solubility in aqueous ethanol, and its inertness to testicular hyaluronidase (*2*). Desulfated B was differentiated from desulfated A by its resistance to the bacterial hyaluronidases. All the chondroitin sulfates show molar ratios of unity of uronic acid-to-N-acetylgalactosamine-to-sulfate when the uronic acid is determined by the orcinol colorimetric (*3*) or the Tracey CO_2 (*4*) method. However, the carbazole colorimetric value (*5*) of B is markedly lower. The low value is retained in the desulfated product and also in fractions of low molecular weight obtained after acid hydrolysis.

The five available uronic acids or their lactones were investigated, and three yielded low carbazole values (Table 1), indicating that a uronic acid other than glucuronic may be present in B.

Chondroitin sulfate B (carbazole-to-orcinol ratio = 0.41) was hydrolyzed on the steam bath in the presence of Dowex 50 (H^+). The products of hydrolysis were fractionated on a Dowex 1 × 10 (acetate) column with increasing concentrations of acetic acid. The first acidic fraction removed was a mixed disaccharide fraction that was separated

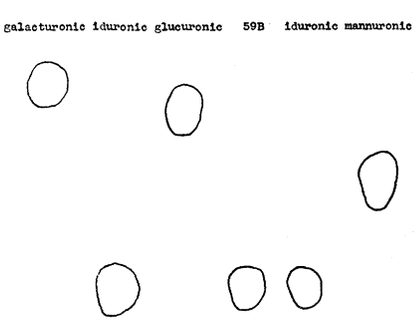

Fig. 1. Trace of a paper chromatogram of the uronic acids and of sample 59B isolated from chondroitin sulfate B. Guluronic acid (not shown) has a mobility similar to that of glucuronic acid in this solvent system (*7*).

Table 1. Colorimetric uronic acid values of the uronic acids or their lactones and fractions isolated from the Dowex-1 column. D-Glucurone was used as a standard.

Compound	Carbazole	Orcinol	Carbazole/orcinol ratio
D-Glucurone	100	100	1.0
L-Gulurone	32	106	.30
L-Iduronic acid	29	130	.22
D-Mannurone	17	128	.13
D-Galacturonic acid	120	127	.95
57B	21	66	.32
59B	41	92	.45
Disaccharide I	12	53	.23
Disaccharide II	33	29	1.2

preparatively by paper chromatography (butanol, acetic acid, and water; 50:12:25) into disaccharides I and II. Disaccharide I, the major disaccharide product, yielded a carbazole-to-orcinol ratio similar to that obtained from iduronic acid (Table 1), and a hydrolyzed sample produced a spot on a paper chromatogram with an R_f value identical to that of iduronic acid. The disaccharide has recently been obtained in crystalline form and is under investigation. It was similarly shown that disaccharide II contains glucuronic acid as its uronic acid moiety. Both disaccharides contained galactosamine as the only demonstrable hexosamine present under the conditions of the ninhydrin oxidation method (*6*).

The next acidic fraction from the Dowex-1 column was a sirup. It was passed through an Amberlite IRC 50 (Na^+) column and freeze dried as the crude sodium salt. Samples 57B and 59B (Table 1) were obtained in two different runs. The reducing values indicated that these were monosaccharides. Paper chromatography (Fig. 1) revealed a spot with an R_f value equal to that of crystalline L-iduronic acid. A trace spot with an R_f value equal to that of glucuronic acid was also present, and this accounts for the difference in carbazole-to-orcinol ratios of the isolated acid and synthetic iduronic acid.

Aqueous solutions of the free uronic acids or their lactones, with the exception of galacturonic acid, which does not form a lactone, each showed two spots on paper chromatograms due to acid-lactone equilibria. The isolated uronic acid and the synthetic iduronic acid had identical R_f values for both acid and lactone in four different solvent systems. Identical mobilities of the isolated and synthetic acids were also obtained on paper electrophoresis in 0.1*M* borate buffer (*p*H 9.4) and in 0.1*N* acetic acid.

This appears to be the first isolation of iduronic acid from a biological source and of a uronic acid other than glucuronic acid from an acid mucopolysaccharide. However, there is an interesting parallel in the isolation of L-guluronic acid from alginic acid (*8*), for L-guluronic acid is the C-5 epimer of D-mannuronic acid, the same relationship that L-iduronic acid bears to D-glucuronic acid (*9*).

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Anomalous Carbon-14 Content of Carbon Dioxide from Sewer Gas

During a study of the carbon-14 content of atmospheric carbon dioxide, an attempt was made to use sewer gas as a source of modern carbon. In the course of this work, an anomalous, high content of C^{14} in the CO_2 fraction of this gas has been found.

Sewer gas was piped directly into our laboratory from the adjacent sewage-treatment plant that processes sewage from the District of Columbia. The plant employs primary treatment followed by anaerobic digestion. The gas from the digestion process consists principally of methane (65 to 70 percent) and carbon dioxide (30 to 35 percent) with low percentages of nitrogen, oxygen, carbon monoxide, and other gases. A similar material was used in the first identification of C^{14} in nature (*1*).

Carbon dioxide samples were obtained by passing the sewer gas through a sodium hydroxide solution. The remainder of the CO_2 was removed by suitable absorbers, the CO_2-free methane was converted to CO_2 by passage over hot copper oxide, and the CO_2 was absorbed