Preservation of Monkey Testicular Tissue with Human Albumin.* (20197)

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During the course of studies designed to obtain multiplication of human fibroblasts in roller tubes, human and bovine albumin were substituted for horse serum in the culture medium. As a result of these experiments three observations were made. 1) Outgrowth of fibroblasts occurred in a medium consisting of 1.25% human albumin, Simms-Hanks' solution, and chick-embryo extract. 2) Tissues grown in media containing varying quantities of unheated horse serum became granular and showed evidence of deterioration in less than two weeks. These disintegrative changes were prevented by adding albumin. 3) When 10% albumin was employed, the original tissue in the roller tube did not proliferate but appeared unusually well preserved after incubation at 37°C for one month. These facts stimulated interest in the use of albumin for maintaining tissue in a viable form and as a constituent of culture media.

Method. The method of roller tube culture was essentially the same as that already reported(1). Commercial lots of 25% salt-poor human albumin in buffered diluent were kindly furnished by Squibb and by Cutter Laboratories. Albumin preserved with merthiolate is toxic for fibroblasts. Chick-embryo extract was prepared after the method recommended by Youngner *et al.*(2).

Experimental. A single testicle from an immature rhesus monkey was minced into pieces suitable for roller tube cultures. Portions of the tissue were embedded and incubated the same day. The remainder was placed in the refrigerator in 50 ml of Hanks' salt solution which contained 2.5% human albumin, and streptomycin and penicillin in concentrations of 0.1 mg and 100 units respectively. Six weeks later pieces of the tissue were removed, washed and embedded in chick plasma clots. The culture medium consisted of 1.25% human albumin, 10% chick-embryo extract, and 10% unheated horse serum, in Simms-Hanks' mixture,† and the usual concentration of penicillin and streptomycin. The same process was repeated after the fragments had remained in the refrigerator for 7 and 8 weeks. The cultures were examined daily and the experiment was terminated when the tissue explants showed a well marked zone of fibroblastic proliferation measuring 1-2 mm. Initial growth was observed within 2 days in some fragments; by 4 days proliferation was visible grossly, and in one week or less the zone of cell outgrowth had reached 1.5 to 2 mm. Good outgrowth of fibroblasts was obtained from practically all fragments despite the fact that the minced testicle had been held in the refrigerator for as long as 8 weeks.

In a second experiment monkey testicular tissue was minced and divided into 2 portions. One was refrigerated in 25 ml of Simms-Hanks' solution and the other was placed into an equal amount of Hanks' balanced salt solution containing 2.5% human albumin. The results of tests for viability, after storing testicular tissue in the refrigerator for a total of 5 weeks, are shown in Table I. The specimens kept in albumin for 5 weeks began to grow in 3 days and reached 1-2 mm by 6 days. The fragments held in Simms-Hanks' solution remained viable for only two weeks.

Human albumin was also incorporated in culture media and the results are presented in Table II. Freshly obtained monkey testicle was grown in varying quantities of 50% chick-embryo extract and human albumin, and made up to proper concentration with Simms-Hanks' solution. Rapid and adequate multiplication of monkey fibroblasts took place over a wide range of albumin or chick-embryo extract concentrations in the absence of horse serum. A minimum of 10% chick-embryo was required and a concentration of 1.25%

* Aided by a grant from the National Foundation for Infantile Paralysis.

† 1 part Simms' serum ultrafiltrate and 3 parts Hanks' balanced salt solution.

TABLE I. Preservation of Monkey Testicular Tissue in 2.5% Human Albumin.

Refrigeration, wk	Growth after 10 days at 37°C: Simms-Hanks' solution, Tubes 1	2	3	4	5	6	2.5% human albumin, Tubes 1	2	3	4	5	6
1	4/8	3/6	3/6				8/8	8/8	8/8			
2	0/6	1/8	2/6	1/6	2/6	5/8	6/6	6/6	7/7	6/6	8/8	7/7
3	0/6	0/5	0/5	0/7			4/4	8/9	6/6	8/8		
5	0/6	0/8	0/7	0/8	0/6	0/7	5/5	4/4	6/6	8/9	5/6	5/5

Denominator = total fragments; numerator = No. growing.

TABLE II. Effects of Varying Quantities of Human Albumin and Chick-Embryo Extract on Growth of Monkey Fibroblasts.

Medium	% albumin	% CEE	Fragments growing, Tubes 1	2	3	Degree of growth	Initial & final pH
1			9*	9	10	+	7.5
	1.25	25	10	10	10	4+	6.9
2			10	10	10	+	7.6
	1.25	10	10	10	10	4+	7.0
3			10	10	8	+	7.6
	1.25	5	10	10	10	4+	7.0
4			4	8	8	±	7.6
	1.25	1	10.	10	10	2+D	7.2
5			0	4	3	±	7.6
	1.25	0	0	10	9	+D	7.2
6			0	0	0	—	6.8
	7.5	10	9	10	10	+	7.0
7			4	6	6	±	7.0
	5.0	10	10	10	10	3+	7.0
8			9	9	10	+	7.3
	2.5	10	10	10	10	4+	7.1
9			10	10	9	+	7.5
	1.25	10	10	10	10	4+	7.1
10			9	9	8	±	7.9
	.25	10	10	10	10	4+	7.0
11			6	10	9	±	8.0
	0	10	10	10	10	2+D	7.1
12			4	6	8	+	7.7
	1.25	10†	10	10	10	4+	7.0
13			10	9	10	+	8.0
	0	10†	10	10	10	3+D	7.0
14			8	6	4	±	8.1
	0	0	10	10	10	2+D	7.2

CEE = 50% chick-embryo extract.
* No. indicates growth of fibroblasts from total of 10 explants.
† 10% unheated horse serum added.
± Early proliferation of single fibroblasts.
+ = Definite outgrowth from numerous margins of the explants.
2+ = ½ mm zone of fibroblasts; 4+ = 1-2 mm zone.
First reading after 2 days incubation at 37°C; second reading after 8 days. Final pH obtained after 8 days incubation at 37°C.
D = Disintegrative signs as rounding of cells, granularity.

albumin was sufficient for good proliferation of cells. The pH was not particularly critical unless extremes were reached (media 6, 11, and 14). In a duplicate experiment, made at the same time, the pH of media 6, 7, 8 and 9 was adjusted with sodium carbonate to 7.4 and growth of fibroblasts was obtained in the presence of 7.5% albumin, but not to the same degree as with the lesser amounts. Changes in pH during growth did not appear

to influence the development of disintegrative changes.

Discussion. The data presented are of interest from the point of view of preservation of monkey testicular tissue in the refrigerator for prolonged periods of time (5 to 8 weeks). This fact should prove helpful in those laboratories where such tissues are used for the isolation and identification of poliomyelitis viruses. The mechanism of action of albumin as a protector of fibroblasts has not been investigated. An analogous situation is the stimulating effects of either whole serum or the albumin fraction on growing tubercle bacilli as originally observed by Youmans and confirmed by others(3-6). In this instance the albumin was thought to neutralize toxic fatty acids and other substances(3,4). To the authors' knowledge human albumin has not been previously used as a constituent of media for the cultivation of cells. Sanford, *et al,*(7) have recently reported an unsuccessful attempt to fortify a medium containing chick-embryo extract and horse serum ultrafiltrate with 6% crystalline bovine albumin. This mixture was not better than whole horse serum for the quantitative cultivation of the L strain of mouse cells. We have utilized mixtures of bovine albumin and horse serum for growing human fibroblasts. Proliferation was equal to that seen with human albumin but degenerative changes appeared earlier.

Summary. Minced immature rhesus testicular tissue remained viable for a period of 8 weeks when refrigerated at 5°C in Hanks' basic salt solution containing 2.5% salt-poor human albumin. Some observations on the use of human albumin as a constituent of tissue culture media have been recorded.

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Received February 5, 1953. P.S.E.B.M., 1953, v82.

Thyroid and Vitamin B_{12} Interactions in the Mouse.* (20198)

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Low doses of thyroid-active substances have been shown to significantly increase the rate of growth in young mice for limited periods of time(1-3). Daily injections of .01-.03 mg of crystalline thyroxine sodium or feeding .04-.32% of iodinated casein in the ration for a 5-week period increased body weight gains by about 28% over untreated control mice(2). This was accompanied by an increase in tissue protein and water, a decrease in fat and greater body length. Excessive doses of thyroid-active materials however, inhibit body growth in young mice, an effect which can largely be counteracted by supplementing the diet with "animal protein factor" or vit. B_{12} (4,5). It was of interest therefore, to determine in mice the interactions betwen a dose of a thyroid-active substance which would increase growth rate and vit. B_{12}.

Methods. A total of 36 immature, male Rockland mice and 45 immature, male Car-

* Part of these data appeared in *Fed. Proc.,* 1951, v10, 91.

† These studies aided in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

‡ Published with approval of the Director of the Michigan Agr. Exp. Station as Journal Article No. 1452.