inactive both in regard to its ketogenic activity and its ability to maintain the life of adrenalectomized rats.

Conclusions. An active adrenal cortex extract has been found to increase the ketosis of fasting female rats when administered in large doses. The ketosis of fasting male rats is only slightly increased. Adrenal cortex extract abolishes the partial oxidation of B-hydroxybutyric acid when fed to fasting rats.

8902 C

Effect of Allantoin Upon Fibroblasts from Cardiac Explants in Tissue Culture.

MARY E. SHIPP AND DUNCAN C. HETHERINGTON. (Introduced by F. H. Swett.)

From the Department of Anatomy, Duke University School of Medicine.

It is difficult surgically and frequently impossible to eliminate all necrotic tissue and purulent materials from some deep wounds such as those of osteomyelitis.[1] To cope with this situation Baer[2] introduced the living maggot treatment which brought about early and complete healing of infections that had been resistant to other forms of accepted therapy. The beneficial results are possibly due to a combination of factors: (1) the maggots feeding upon and thus thoroughly removing all the diseased tissue; (2) the ingestion and physical removal of the microorganisms, with subsequent sterilization of the wound; (3) the proteolytic activity of the maggots' enzymes breaking down the discharge and slough of the wounds into their end products; (4) the maggots crawling about in the wound, causing sufficient irritation to stimulate rapid growth.[3] In addition to these possibilities, it was believed that the larvae secreted some substance which stimulated directly the healing process.

Following investigations of the secretion of maggots, a substance was found which had the property of stimulating healing in infected wounds.[4] It was identified as allantoin, the principal terminol product of purine metabolism in animals below man. It seemed evident that the secretion of this substance into the wounds contributed to the remarkable healing effects obtained by maggot therapy; how-

1 Meyers, J., and Czaja, L. M., *Illinois Med. J.*, 1931, **60**, 124.

2 Baer, William S., *J. Bone and Joint Surg.*, 1931, **13**, 438.

3 Buchman, Joseph, *Ann. Surg.*, 1934, **99**, 251.

4 Robinson, William, *J. Parasit.*, 1935, **21**, 354.

ever, allantoin, alone, could not be substituted for the living larvae. In its new rôle, as a stimulator of tissue growth where development is inactive, allantoin and some of its related substances appear to be more than waste products only. It was suggested that these substances might be used normally in the nuclear structure of the cell.[5]

The following experiment was devised and conducted to determine the effects of allantoin upon the growth and the rate of growth of cells in tissue culture.

All the cultures were made by the cover-slip-hanging-drop method with observance of aseptic precautions during all manipulations. All water used was triply distilled in a pyrex glass apparatus. The plasma was obtained by centrifuging blood drawn, without the use of anticoagulants, from the wing veins of young hens. The embryo juice (25%) for the control cultures was prepared by extracting 7- or 8-day chick embryos in Tyrode solution (pH 7.5 to 7.6) containing 0.25% dextrose. For the test series allantoin, in quantities sufficient for a final dilution of 0.5%, was added to an aliquot part of adjusted Tyrode solution before the extraction of the embryos. This concentration of allantoin was the same as that used in the clinical treatment of deep infected wounds, as reported by Robinson.[4] Heart tissue of 7- or 8-day chick embryos was planted in a mixture of equal parts of embryo juice, containing the allantoin, and plasma. An equal number of controls using the same plasma, stock embryo juice, and embryo heart, were planted at the same time with each series of allantoin cultures. All cultures were incubated at 37°C. and a daily record was kept of each as long as it remained alive. From these data on the control and allantoin cultures, average percentage death rate curves were constructed. With the aid of a delineascope and planimeter the area of the original explant and area of daily outgrowth of each culture were measured and the results statistically treated. (Table I.)

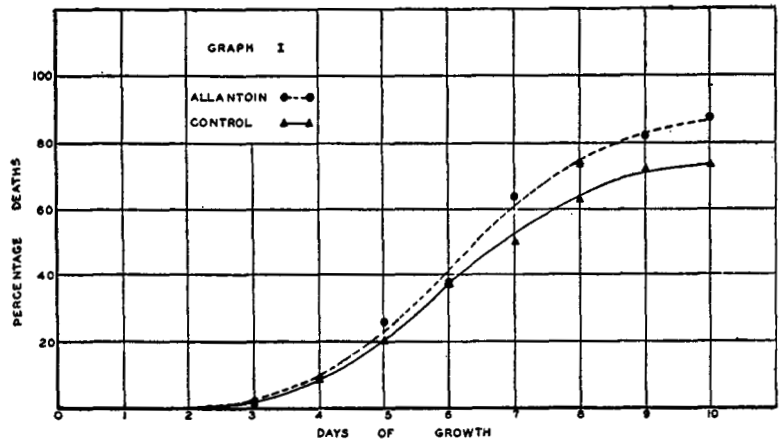
Microscopical study of each of the 560 cultures showed no marked difference in behavior or change in structure of the cells in either the control or experimental series. According to Graph 1, the death rate curves of both series of cultures followed practically the same course for the first 6 days, after which the curve of the allantoin series indicated a definitely greater death rate. This is in accord with the observation from Graph 2 that after the 5th day the growth of cultures in the allantoin medium was slightly increased and with increase in cell population nutrient materials were correspondingly diminished leading to an increased death rate.

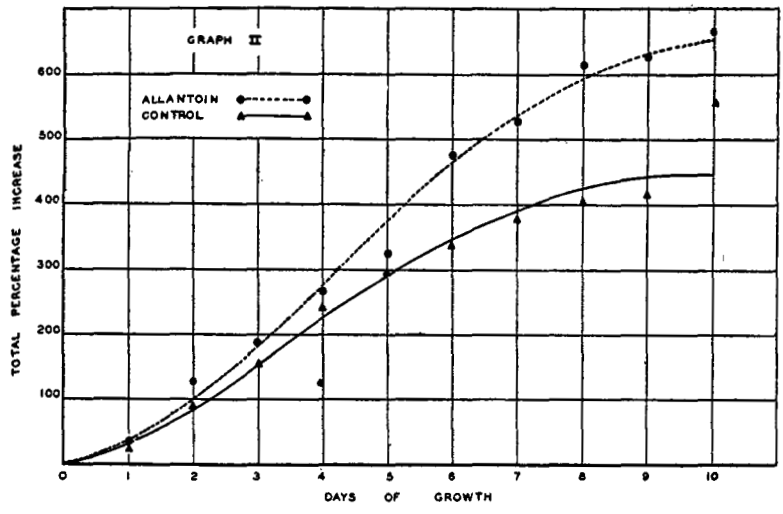
[5] Robinson, William, *J. Bone and Joint Surg.*, 1935, **17**, 267.

TABLE I.

	Allantoin 0.5% Series									Control Series					
Days Growth	Mean Total Area sq.mm.	Probable Error of Mean	Standard Deviation	No. of Cultures in Exper.	Total Growth %	Total Deaths %	D—Difference of Means	σ_D—Standard Error of of Difference of Means*	Significant Difference of Growth $D>3\sigma_D$	Mean Total Area in sq. mm.	Probable Error of Mean	Standard Deviation	No. of Cultures in Exper.	Total Growth %	Total Deaths %
0	4.21	±0.061	± 1.46	270	0	0	1.00	0.17	1.00>0.51	5.21	±0.094	± 2.45	280	0	0
1	5.64	±0.088	± 2.07	270	34	0	0.82	0.21	0.82>0.63	6.46	±0.108	± 2.78	280	24	0
2	9.67	±0.196	± 4.48	270	129	0	0.23	0.38	0.23<1.14	9.90	±0.182	± 4.50	280	90	0
3	12.16	±0.274	± 6.40	264	189	2.2	1.07	0.53	1.07<1.59	13.23	±0.256	± 6.31	275	152	1.7
4	15.33	±0.351	± 8.03	247	264	8.5	2.39	0.71	2.39>2.13	17.72	±0.334	± 7.99	255	240	8.9
5	17.86	±0.472	± 9.50	199	324	25.9	2.79	1.01	2.79<3.03	20.65	±0.512	±11.31	223	296	20.3
6	24.37	±0.735	±14.11	167	478	31.8	1.73	1.48	1.73<4.44	22.64	±0.729	±13.27	175	334	37.5
7	26.45	±0.951	±13.93	97	538	64.0	1.62	1.84	1.62<5.52	24.83	±0.803	±13.96	139	376	50.3
8	30.02	±1.577	±17.52	65	613	75.9	3.86	2.68	3.86<8.04	26.16	±0.984	±14.92	105	402	62.5
9	30.50	±1.878	±16.42	48	624	82.2	3.79	3.18	3.79<9.54	26.71	±1.440	±18.69	78	412	72.1
10	32.19	±2.037	±20.92	35	644	87.0	2.02	4.08	2.02<12.24	34.21	±1.380	±17.67	74	556	73.5

*See foot-note 1.





In Graph 2 it is also apparent that the trend of the growth curve for the allantoin series is noticeably away from that of the controls yet analysis of the data* from which the curves are derived

* Significant difference of growth was determined for any given day from the following formulæ:

$$\sigma_D = \sqrt{\frac{\sigma_1^2}{N_1} + \frac{\sigma_2^2}{N_2}} \text{ and } D > 3\sigma_D$$

where for any one day σ_1 and σ_2 are the standard deviations of the control and allantoin series respectively and N_1 and N_2, the number of cultures; "D" represents the difference of the means and σ_D is the standard error of the difference.[6]

[6] Mills, *Statistical Methods*, H. Holt & Co., 1930, p. 558.

indicates that at no time is the difference in growth rate of the 2 series sufficiently great to have a real significance.

Conclusion. Allantoin stimulates slightly but not to a significant degree the growth of fibroblasts from cardiac explants in tissue culture.

8903 C

Intramuscular Injection of Ascorbic (Cevitamic) Acid and Excretion in the Sweat.

ALFRED LILIENFELD, IRVING S. WRIGHT AND ELIZABETH MACLENATHEN.

From the Second Medical Division (Cornell) of Bellevue Hospital Service, New York City.

The value of the oral and intravenous methods of administration of cevitamic acid has been well established in the treatment of vitamin C undernutrition.[1-9] In order to determine the efficacy of the intramuscular route, a group of patients was given the substance by this method, and the cevitamic acid values of the blood and urine were followed. The patients selected were of varying ages and degrees of vitamin C saturation and were all afebrile. The solution used was made up by mixing one mole of cevitamic acid with one mole of sodium hydroxide and contained 50 mg. of the vitamin per cc. The pH is about 6.3. It has remained stable during the 3 months of the duration of this experiment.* The use of sodium bicarbonate for this purpose, just preceding injection, was suggested by Fisher and Leake.[10]

The cevitamic acid content of the urine of the 2 preceding 24-hour

1 Schultzer, P., *Lancet*, 589, Sept. 9, 1933.

2 Schultzer, P., *Acta Med. Scand.*, 1934, **81**, 111.

3 Schultzer, P., *Acta Med. Scand.*, 1934, **83**, 544.

4 Schultzer, P., *Acta Med. Scand.*, 1934, **83**, 555.

5 Schultzer, P., *Acta Med. Scand.*, 1935, **85**, 563.

6 Wright, I. S., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 475.

7 Wright, I. S., and Lilienfeld, A., *Arch. Int. Med.*, 1936, **57**, 241.

8 Dalldorf, G., and Russell, H., *J. A. M. A.*, 1935, **104**, 1701.

9 Van Eekelen, M., Over opname, Verbruik in Vitscheiding Van Vitamine C Door De Mens, Drukkerj. Fa. Schotanus and Jens, Utrecht, 1936.

*Prepared and supplied through the courtesy of Merck and Co., Inc., Rahway, N. J.

10 Fisher, B. H., and Leake, C. D., *J. A. M. A.*, 1934, **103**, 1556.