TABLE 1

COMPARATIVE ACTIVITY OF α-LIPOIC ACID AND PROTOGEN IN THE GROWTH OF *Tetrahymena geleii* E.

Acetate (%)	Protogen (unit/ml)	Count at 120 hr	Final pH
	1	83,200 ± 12,640*	6.9
	0.5		3.9
0	.25		3.8
	.125		3.5
	0.000		3.4
	1	83,200 ± 5,880	7.3
	0.5		7.2
0.05	.25		6.3, 4.5†
	.125		4.1‡
	0.000		5.3
	1	97,200 ± 14,520	7.5
	0.5		7.5
0.10	.25		7.2
	.125	47,440 ± 1,988	6.2
	0.000		6.0
	α-Lipoic (γ/ml)		
	0.08	95,000 ± 13,520	7.1
	.04		7.4
0	.02		7.4
	0.01	114,800 ± 16,840	7.2
	0.08	111,600 ± 11,880	7.5
	.04		7.5
0.05	.02		7.5
	0.01	131,200 ± 11,200	7.4
	0.08	114,000 ± 8,690	7.6
	.04		7.7
0.10	.02		7.5
	0.01	127,200 ± 10,320	7.6

* This count represents both live and dead animals, since many of the cultures had begun to die here after 72 hr.

† Approximately one half of the cultures had precipitated after 96 hr. Tubes containing the precipitate exhibited the lower pH.

‡ All these cultures had precipitated after 96 hr. Upon microscopic examination, the precipitate was found to consist only of dead cells.

completely in the growth of *Tetrahymena* (Fig. 1). In concentrations ranging from 0.01 to 0.08 γ/ml, α-lipoic acid is more active than protogen in acetate-replacing properties. As noted by Kidder *et al.* (*8*), protogen is active in sparing acetate. Low levels of protogen (0.125–0.5 unit/ml) resulted in considerable acid production in media containing 0–0.05% acetate. Lower population densities were also obtained (Table 1). Concentrations of protogen at 10 units/ml were found by the author (*9*) to be slightly inhibitory in media with 0.1% acetate. Maximal growth (approx 120,000 animals/ml) was obtained by the use of α-lipoic acid in media containing 0.05–0.1% acetate. Considerably lower population densities (approx 80,000 animals/ml) resulted from the use of protogen at the 0.05% acetate level.[4]

[4] Since this paper has gone to press, it has been found that half-maximal growth can be obtained by means of 0.0005 μ of α-lipoic acid.

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The Effects of Cortisone and Heparin on Eosinophils in Defibrinated Human Blood [1, 2]

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Four hours after injection of corticotropin (ACTH) into healthy individuals the blood eosinophil count is found to be significantly reduced from pre-injection levels. This adrenocortical eosinopenia also develops in man after treatment with 17-hydroxycorticosterone (Compound F), corticosterone (Compound B), and cortisone, but the latter steroid does not have as striking an eosinopenic effect as the other hormones. During the past 3 years we have tried to reproduce adrenocortical eosinopenia *in vitro* by incubation of human blood with cortisone. Baldridge *et al.* (*1*) and other investigators have also tried to do this, but without success. Both Baldridge and ourselves used heparin as an anticoagulant for blood samples.

Recently we incubated, under sterile conditions, defibrinated blood from 15 patients ill with various diseases associated with eosinophilia. They included patients ill with asthma, polyarteritis nodosa, and other diseases. Incubation of their blood for 4 hr at 37.5° C, alone, with saline, or with nonsteroidal microcrystals (e.g., sulfonamides), had only slight effects on the level of eosinophils in the sample. On the other hand, when a commercial microcrystalline suspension of cortisone acetate or pure crystalline cortisone acetate (1 mg/3 ml blood) was mixed with the defibrinated blood, the absolute eosinophil counts were significantly reduced after 4 hr incubation. This effect of cortisone on the eosinophil count could be blocked by adding heparin (40 u) to the defibrinated blood before introducing the cortisone. The data from our experiments are shown in Table 1. In some experiments with Compound F we have found that it can also produce significant eosinopenia when incubated with defibrinated blood.

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[2] We wish to thank Elmer Alpert, of Merck & Co., Inc., Rahway, N. J., for supplying the pure cortisone acetate, and Curtiss E. Meyer, of the Upjohn Company, Kalamazoo, Mich., for supplying the Compound F used in this study.

The technique of counting eosinophils used in our laboratory and the statistical treatment of the data obtained have been described in detail elsewhere (*2–5*). In the investigation reported here each absolute eosinophil count was obtained by counting cells taken up into 2 standard leucocyte pipettes and delivered into 4 double Levy counting chambers. The data obtained were highly significant for each of the bloods studied, as well as for difference of means, P being less than 0.001.

TABLE 1

EFFECT OF INCUBATING DEFIBRINATED HUMAN BLOOD*

Statistical data	Fall of absolute eosinophil counts in defibrinated human blood after 4 hr incubation (%)		
	Saline	Cortisone acetate	Heparin plus cortisone acetate
Mean	15.2	44.9	11.4
S. E.	4.4	3.6	2.7
Fiducial limits ($P = 0.05$)	5.1–25.3	37.2–52.6	5.5–17.3
Mean $\pm \sigma$	1.9–28.5	30.8–59.0	2.1–20.7

* Blood from 15 patients with eosinophilia (490–3000 cells/mm³) was incubated for 4 hr at 37.5° C. Percentage fall is in absolute eosinophil counts from zero time, when saline, cortisone acetate, or heparin, followed by cortisone acetate, was added to the blood.

Thrombosis is a common complication of therapy with ACTH and cortisone acetate (*6,7*), and it also develops after surgical procedures, when adrenal hyperactivity, eosinopenia (*8*), and reduced blood heparin tolerances (*9*) are found. Therefore, on the basis of our findings, it is tempting to speculate that in man adrenocortical hormones interact with heparin as they do with other polysaccharides, such as joint hyaluronic acid (*10*). However, the concentrations of cortisone and heparin used in the *in vitro* investigations reported here were very much larger than obtain in clinical practice.

The morphological changes in the eosinophils which appear after *in vitro* incubation with cortisone, and the effects of combining heparin and adrenal steroid therapy in man, will be reported elsewhere.

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An Implantor for Forming and Accurate Placing of Small Pellets

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Previously reported techniques for implanting solids into animals (*1–3*) were for large, preformed pellets. In the course of experiments requiring accurate placing of a known small amount of compact, sterile solid, an instrument was designed and constructed which fulfilled these requirements.

The device (Fig. 1), made entirely of stainless steel,

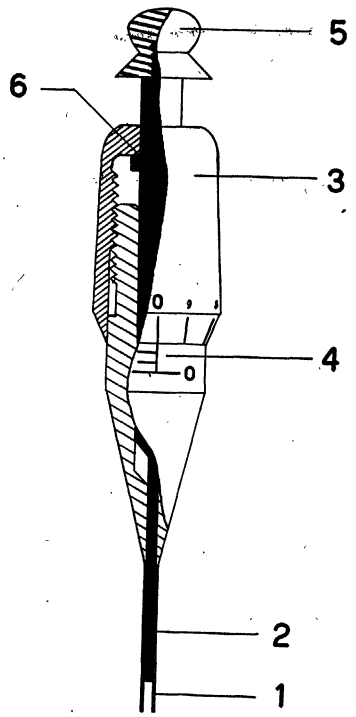

FIG. 1.

consists of a thin-walled tube (1), with a close-fitting plunger (2). When preparing a pellet of material for implantation, the plunger is set to the desired length by turning the body (3) to the setting on scale (4). The plunger head (5) is withdrawn to collar check (6). The body of the device is held firmly, and the tube tamped into a small dish containing the substance (fine crystals or powder) to be used for study.

A sharp needle approximately the size of the pellet is used to make an entry for the tip of the instrument, after which the solid which has been compacted in the end of the tube is ejected *in situ*. Sterile precautions in preparation of the pellets consisted of using sterilized glassware and implantor, with care to avoid contamination, and were sufficient to avoid infecting experimental animals.

The number of taps required for compacting a