None of the controls showed spontaneous recovery of the extinguished habit during the 3-week period in which the experimentals evidenced reinstatement.

Despite the fact that the nature of the habit acquired by our subjects and the method used to induce the seizures differed from Gellhorn's, it seems apparent that convulsive states that include coma are most effective in the reinstatement of a conditioned fear response. However, in the case of reinstatement of the response used in this study, it seems that excitement produced by treadmill running does at least have a transient effect.

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Relationship of Nitrogen Content of Hyaluronic Acid Preparations to Hyaluronidase Activity

Eighteen samples of hyaluronic acid were prepared (*1*) from various tissues by the methods of Harris and Harris (*2*), Tolksdorf *et al.* (*3*), Meyer (*4*), and Dorfman and Ott (*5*). Tissues employed were human umbilical cords, spleens, carcinomatous uteri, a portion of greater omentum invaded by carcinoma, a carcinomatous breast, pooled CBA mouse mammary tumors, pooled rat tumors (Walker 256), and pooled vitreous humor from beef eyes. Variations in the products from the same and different tissues are apparent in Table 1.

Turbidity was determined in the Coleman photonephelometer (Model 7) by a technique modified from that of Kass and Seastone (*6*). Aliquots (0.2 mg in 0.1 ml) of each of the hyaluronic acid preparations were placed in separate Wassermann tubes (13 by 100 mm). To each of these tubes was added 0.1 ml of acetate-buffered sodium chloride. After mixing, the tubes were incubated at 37°C for 15 minutes. Acidified albumin (7.0 ml) was added to each of the tubes, which were then incubated at room temperature for 20 minutes. The contents of each tube were poured into a cuvette and read in the photonephelometer adjusted to maximum sensitivity. To a second series of tubes containing the different substrates was added 0.1 ml of buffer solution containing 5 turbidity-reducing units of bull testis enzyme (Wyeth). This set of tubes was treated in the same manner as the controls. The effect of the given concentration of hyaluronidase on each of the hyaluronic acid preparations was determined by subsequent decrease in turbidity.

The percentage of nitrogen (duplicate samples of 1.0 mg) of the hyaluronic acid preparations was determined by the micro-Kjeldahl method as described by Kabat and Meyer (*7*).

Turbidities varied with different hyaluronic acid-hyaluronidase mixtures. A direct relationship was noted between the percentage of nitrogen of the hyaluronic acid preparations and the degree of activity of bull testis enzyme on the substrate. An enzyme activity ratio (EA ratio) was obtained for each substrate by dividing (i) the photonephelometer reading given by 0.2 of hyaluronic acid by (ii) the photonephelometer reading given by 0.2 mg of hyaluronic acid after it had been acted on by 5 turbidity reducing units of bull testis enzyme for 15 minutes.

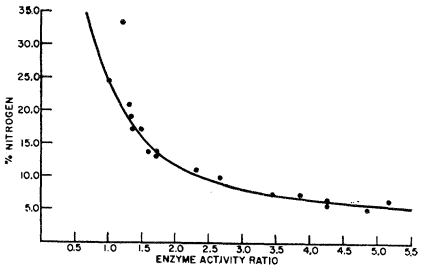

Fig. 1. Relationship of enzyme activity ratio of hyaluronic acid substrates to their nitrogen content.

Table 1 lists the laboratory number, tissue source of substrate, yield, EA ratio, and nitrogen content for each of the preparations. Hyaluronic acid preparations presenting low EA ratios are associated with high percentages of nitrogen and, inversely, those preparations yield-

Table 1. Hyaluronic acid preparations. Italic numbers in parentheses in column 1 refer to literature that describes the method of preparation.

Preparation	Tissue		Yield (g)	Galvanometer readings 0.2 mg substrate		EA ratio	Content (%N/mg)
	Source	Wt. (g)		No enzyme	5 TRU		
1 (*2*)	Human umbilical cord	100*	1.7	135	51	2.65	9.8
2 (*3, 5*)	Human umbilical cord	50*	18.0	34	8	4.25	6.5
3 (*3, 5*)	Human umbilical cord	2†	0.84	31	6	5.15	6.5
4 (*3, 5*)	Human umbilical cord	100*	4.75	92	19	4.85	5.1
5 (*3, 5*)	Cow carcinoma‡	89 → 4.5 →	0.05	135	101	1.33	19.1
6 (*3, 5*)	Cow carcinoma‡	89 → 5.0 →	0.28	81	19	4.25	5.9
7 (*3, 5*)	Human spleen (normal)	24* → 2.7 →	0.16	6	5	1.2	33.3
8 (*3, 5*)	5 human spleens	99* → 1.3 →	0.07	149	147	1.0	24.5
9 (*3, 5*)	Pooled rat tumors (Walker)	25*	2.0	111	87	1.3	20.8
10 (*3, 5*)	§	0.5	0.037	116	85	1.36	17.1
11 (*3, 5*)	§	0.5	0.033	90	61	1.47	17.1
12 (*3, 5*)	Human uterine adenocarcinoma	32* → 4.0 →	0.5	44	28	1.57	13.8
13 (*3, 5*)	Human uterine adenocarcinoma	20* → 5.6 →	0.5	60	4	15.0	14.1
14 (*3, 5*)	Human umbilical cord	90*	5.4	112	29	3.87	7.3
15 (*3*)	Human mammary adenocarcinoma	177‖	0.3	24	7	3.43	7.5
16 (*3*)	Human omental adenocarcinoma	398‖	3.8	36	21	1.71	13.2
17 (*3*)	CBA mouse mammary tumors (pooled)	46‖	0.34	27	16	1.7	13.8
18 (*4*)	Beef eye vitreous humor (pooled)	370‖	0.1	32	14	2.3	11.0

* Dry weight. † A portion of preparation 2 was reprocessed. ‡ Hereford cow squamous cell carcinoma collected by Frank X. Gassner of Colorado A & M College, Fort Collins. About 500 g (wet weight) yielded 89 g of substrate, which was reprocessed in two portions (preparations 5 and 6). § Preparation 9 was reprocessed in two 0.5-g amounts. Results recorded as preparations 10 and 11. ‖ Wet weight.

ing high EA ratios are associated with low percentages of nitrogen (except preparation 13). The index of each of the hyaluronic acid preparations was plotted on arithmetic graph paper as the ordinate (Fig. 1). Laboratory preparations 7 and 13 provided the maximum deviations from the curve. Preparation 13 gave an unusually low turbidity reading after having been acted on by 5 turbidity reducing units of bull testis enzyme. It is doubtful whether preparation 7 actually was a hyaluronate. Certain actions of hyaluronidases of varying origin on these substrates will be reported at length.

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Foot-and-Mouth Disease Virus: Its Growth and Cytopathogenicity in Tissue Culture

We wish to report that foot-and-mouth virus (FMV) reproduces and causes cytopathogenic changes in both swine and bovine kidney tissue cultures. Practical techniques have been developed for the assay of the virus and its antibodies as well as for the production of virus on a relatively large scale.

Swine or bovine kidney from young animals was cut into pieces weighing approximately 0.5 g and placed in phosphate buffer solution at *p*H 7.3 containing penicillin and streptomycin. The tissue was washed repeatedly in fresh changes of buffer solution and then stirred gently in a 0.25-percent trypsin buffer solution. The liberated cells were decanted at 10-minute intervals through cheesecloth, centrifuged at low speed, washed twice, and diluted 1 to 200 with culture medium. The medium consisted of Hanks' salt solution containing 2.0 percent bovine serum, 0.5 percent lactalbumin hydrolyzate, penicillin, and streptomycin. Culture tubes (16 mm in diameter by 150 mm) and Roux flasks were seeded with the cellular suspensions and incubated stationary at 37°C. The swine and bovine cultures developed confluent outgrowths of mixed epithelial and fibroblastic cells within 3 and 6 days, respectively.

Table 1. Cytopathogenicity and growth of foot-and-mouth disease virus type A in tissue culture.

Passage		Total dilution* (neg. log)	Tissue culture ID_{50}/ml		Generalized FMV disease in
No.	Method†		TP expt. in swine kidney (log)	Titrations in bovine kidney (log)	
1	CP	2.0			
2	CP	3.0			
3	CP	4.0			
4	TP	5.0	5.80		
5	TP	9.0	5.98	6.80	Guinea pigs
6	TP	13.0	5.68	5.68	
7	TP	16.0	‡	5.98	
8	TP	19.0	4.80		
9	TP	21.0	6.15		
10	TP	25.0	5.93		
11	TP	29.0	5.68		
12	TP	31.0	5.98	5.47	
13	TP	39.0	5.80		Steer
14	TP	35.0	6.13		

* Effective dilution of original mouse infectious tissue (neg. log). † CP, conventional passage method; TP, titration-passage technique. ‡ Culture acidity interfered with reading.

Foot-and-mouth virus type A was passed 14 consecutive times in swine cultures (Table 1). The first four passages were made by conventional methods. The virus inoculum for the first passage was of bovine origin and was passed once in suckling mice just prior to its use (*1*). When it was inoculated into cultures, cytopathogenic changes were produced that were similar to those reported for cultures infected with vesicular exanthema and vesicular stomatitis viruses (*2, 3*). Isolated plaques of pyknotic cells were formed in less than 15 hours. Passages 5 to 14 were made by a titration-passage technique, which had been used previously with vesicular stomatitis virus, in which each passage is made as a complete titration (*3*).

Five cultures were inoculated with each dilution passed. After 20 hours' incubation, the cultures were examined microscopically. The pooled fluid from the highest dilution in which all cultures exhibited extensive cytopathogenic damage was serially diluted and used as inocula for the next titration passage. In most instances, this procedure effected a 10^{-3} or 10^{-4} interpassage dilution of the cytopathogenic agent (Table 1, column 3). The inocula used for serial passages 5 to 7 and 12 in swine cultures were also assayed in bovine cultures. Table 1 shows that titration-passage inocula usually contained about $10^{6.0}$ ID_{50} per milliliter for both kinds of cultures. After a few passages in swine cultures, it was evident that infectivity of the original virus inoculum had been diluted out and that the cytopathogenicity that persisted was probably owing to the propagation of FMV.

This presumption was confirmed by animal inoculations and serum neutralization tests. Passage fluid No. 5 (10^{-9} dilution of the original mouse tissue) was inoculated intradermally into the foot pads of guinea pigs. Passage fluid No. 13 was inoculated intradermolingually into a Hereford steer. Both animal species developed extensive local and generalized lesions of foot-and-mouth disease.

In the neutralization tests, the capacities of normal guinea pig serum and of two antiserum samples (*1*) to neutralize the infectious culture fluid from passage No. 5 were determined. The antiserums used were from guinea pigs that had been hyperimmunized to vesicular stomatitis virus (New Jersey type) and to FMV (type A). Tenfold serial dilutions of the infectious fluid were made and aliquots of each were mixed in equal parts with a 1 to 5 dilution of each serum type. The virus and virus-serum mixtures were incubated for 1 hour at 37°C and then assayed in bovine kidney cultures. The virus fluid alone was found to contain $10^{5.83}$ tissue culture ID_{50} per 0.2 ml, but in the presence of the type A antiserum, it retained less than 10 such units of infectivity. The normal and heterologous serums had no detectable neutralizing activity.

It is estimated that the kidneys from one calf will supply enough cells to prepare 50,000 cultures in tubes or more than 300 in Roux flasks. The cultures in tubes are now being used regularly for assays of virus taken directly from in-