17108. Biologic Absorption of Insolubilized Gelatin Films.

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Our experience in preparing absorbable hemostatic sponges from gelatin[1,2] encouraged us to investigate the possibility of developing a gelatin film that might also be clinically useful. During the course of our studies Bing[3] reported his observations using gelatin sponge at the end of which paper he mentionel without elaboration an incomplete study involving a gelatin film preparation.

The purpose of this report is to submit the data we have obtained from studying the *in vitro* water solubility and enzymatic digestion and *in vivo* biologic absorption of uniquely prepared gelatin films.

Several materials, both absorbable and nonabsorbable, have been employed in surgery as films. Brown, *et al.*[4] in their report on the use of polythene film mentioned most of these and noted the limitations of each. Oppenheimer's[5] report suggests that nonabsorbable substances must be observed for a sufficient period to assure that they are not carcinogenic since these investigators noted that cellophane implants induced sarcomas in rats after remaining *in situ* more than 11 months.

Materials and Methods. Films were prepared from commercial pigskin gelatin. To remove cations each 100 g of raw, ground gelatin was eluted for 2 hours with one liter of cold (5°C) M/128 glacial acetic acid; the acid was drawn off and the gelatin mass washed several times with cold double distilled water.

Usually 5% solutions were prepared from this washed gelatin. To these were either added nothing or various small quantities of U.S.P. formalin. The mixture was incubated at 37°C for 2 hours after which films were cast, dried on stainless steel plates under controlled humidity and heated at 140°C for different lengths of time. The investigations which are reported here were carried out on films that were approximately .003 inch thick. Films of such light weight, even when dry, are remarkably pliable and only break when folded sharply.

To obtain sterile preparations, when desired, the gelatin films were subjected to routine dry heat (140°C for 4 hours) sterilization in accordance with the official U.S.P. method.[6]

Three criteria were employed to establish to what degree a given treatment had altered the gelatin molecule yielding films with varying physical and biologic characteristics.

The first of these was the tendency of the film to go into water solution. To uniform quantities of film was added an appropriate volume of distilled water which stood on the gelatin at room temperature overnight. The next morning the solution was heated on a steam bath with occasional stirring and the films observed until dissolved or for at least 24 hours.

In another test the time for *in vitro* digestion[1] of films by pepsin was determined. To pieces weighing 150 mg was added 100 cc of a 1% U.S.P. pepsin solution. The containers were placed in a water bath held at 37°C and mechanically agitated; the time was noted when the last fragment had been digested.

Finally biologic absorption and possible reaction were investigated by implanting pieces that weighed roughly 15 mg (ca. 10 x 12 mm) usually in muscle layers, in the legs of rats using a technique similar to one that has been previously[2] described. Sixteen albino rats weighing around 300 g were prepared in each series.

1 Correll, J. T., and Wise, E. C., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 233.

2 Correll, J. T., Prentice, H. R., and Wise, E. C., *Surg. Gynec. and Obst.*, 1945, **81**, 585.

3 Bing, J., *Acta Pharmacol. and Toxicol.*, 1947, **3**, 364.

4 Brown, M. H., Grindlay, J. H., and Craig, W. McK., *Surg. Gynec. and Obst.*, 1948, **86**, 663.

5 Oppenheimer, B. S., Oppenheimer, E. T., and Stout, A. P., PROC. SOC. EXP. BIOL. AND MED., 1947, **67**, 33.

6 Pharmacopeia of the United States, thirteenth revision, Mack Publishing Co., Easton, 1947.

TABLE I.
In vitro and *in vivo* Evaluations of Absorbable Gelatin Films.

Film preparation				
No.	Treatment	Response in hot water	Proteolytic digestion time	Biologic absorption
B-98-1	Untreated	In solution in 1 to 2 min.	Film soluble	Gone in less than 24 hrs.
B-98-2	Heated 2 hrs	In solution in 7 to 8 hrs.	70 to 80 min.	
B-98-3	Heated 4 hrs	Disintegrated into fragments in 24 hrs.	1½ to 2 hrs.	Gelatinous in 1 day; gone in 4 days
B-93-1	.03% formalin; not heated	In solution in 30 min.	40 min.	
B-93-2	.03% formalin; heated 4 hrs	Much swelling; no solution in 24 hrs.	2 to 4 hrs.	
B-93-3	.03% formalin; heated 8 hrs	Some swelling; no solution in 24 hrs.	5 to 8 hrs.	Gelatinous in 2 weeks; gone in 7 to 8 weeks
B-97-1	.06% formalin; not heated	In solution in 25 min.	60 to 80 min.	
B-97-2	.06% formalin; heated 4 hrs	Some swelling; no solution in 24 hrs.	5 to 8 hrs.	Gelatinous in 2 weeks; gone in 7 to 8 weeks

Results. The data obtained from several different investigations have been organized into a single table (Table I) for presentation. Values are reported in ranges to indicate that the tests cannot be reproduced absolutely from one experiment to the next, but that they do indicate direction. Thus it can be predicted that a film which will finally go into solution in water in 7 to 8 hours (B-98-2) will be digested by pepsin more rapidly than a film which only swells and will not dissolve in water (B-93-2).

It is our experience also that insolubilized films with short enzymatic digestion times (B-98-1, B-98-3) will soften and be resorbed in the body at a faster rate than those with appreciably longer (B-93-3, B-97-2) *in vitro* times.

Enzymatic digestion times and biologic absorption do not, however, have a straight line relationship. For we have observed with gelatin films, as Morrison and Singer have previously noted with fibrin films,[7] that variations in the mode of preparation may result in greatly prolonged *in vitro* digestion times with inconsiderable if any variation in the observed *in vivo* resorption. In experiments not recorded in the table we have prepared insolubilized gelatin preparations which remained undigested by pepsin after 24 hours yet were absorbed biologically in periods of time only slightly longer than films which were insolubilized to a lesser degree according to their *in vitro* characteristics.

Resistance to water solubility was imparted to the film by hot air heating of the dried film (B-98-2, B-98-3) or by the addition of minute quantities of formalin (B-93-1, B-97-1) to the original solution. A combination of the two techniques resulted in a greater degree of insolubilizing (B-93-2, B-93-3, B-97-2). From the table it can be seen that, generally, the more intensive the treatment by either or both of the methods the more altered the film according to the tests that were applied.

Biologic implants of films that had *in vitro* proteolytic digestion times of less than 4 hours softened in the muscle tissue rapidly and no residue could be detected upon gross inspection by the fourth day (B-98-3).

Because it seemed likely that such films would be clinically impractical, preparations were made that were more resistant to *in vitro* digestion. *In vivo* these were found to withstand undue disintegration for 8 to 10 days, to be gelatinous in about 2 weeks and be absorbed in 7 to 8 weeks (B-93-3, B-97-2).

In the series in which gelatin film B-98-3 was implanted in the left thigh muscles a comparable piece of regenerated cellulose (Du Pont cellophane No. 600 PT) was implanted in the same area in the right side. On inspec-

[7] Morrison, P. R., and Singer, M., *J. Clin. Invest.*, 1947, **26**, 929.

tion as long as 11 weeks after implantation the cellophane was readily located encapsulated in a thin membranous envelope; it showed no evidence of disintegration or resorption. Lowry[8] reported a tendency of certain cellophane films which he studied to be absorbed. In the studies of Oppenheimer *et al.*[5] the regenerated cellulose which they implanted was unabsorbed over a period of 11 months or more.[9]

In addition to the gross observations which are recorded here, specimens of the implant areas were fixed and submitted for histologic examination; the results of the histologic readings will be reported in detail in another paper. Since the properties of water insoluble gelatin film suggested that it might have clinical utility wherever an absorbable membrane is indicated, this possibility is being independently investigated in several surgical research laboratories.

[8] Lowry, M. L., *Arch. Surg.*, 1946, **52**, 160.

[9] Oppenheimer, B. S., personal communication, November 1948.

Summary. The preparation and properties of gelatin films, which resisted water solution in varying degrees, have been described.

These films, although water insoluble, have been shown to be digested in different lengths of time when subjected to proteolytic enzymes *in vitro* in a standardized assay.

Some of the altered gelatin films were implanted into muscle areas of albino rats. Upon visual inspection they were found to be absorbed at the recorded intervals, with no evidence of untoward tissue reaction. The data indicate that the biologic absorption time can be roughly predicted from *in vitro* digestion results; as the *in vitro* times become greatly prolonged, however, a comparable resistance to biologic absorption is not seen.

Cellophane implants were not absorbed but at the eleventh week were encapsulated in a thin membranous capsule.

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17109. Sodium Acetate as a Source of Fixed Base.*

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Recent studies have shown that acetic acid is an extremely rapidly oxidized product of the intermediary metabolism of both carbohydrate and fat.[1] From the large turnover of acetate in the normal metabolic processes of the body, it is obvious that sodium acetate should serve as a rapidly available source of fixed base. Using the change of serum CO_2 content as an index of the presumptive rate of oxidation of the acetate ion, studies were performed in dogs and in man to define the usefulness of sodium acetate as a source of fixed base.

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[1] Bloch, K., *Physiol. Rev.*, 1947, **27**, 574.

Procedure and results. Dogs, anesthetized with pentobarbital, were infused intravenously with a solution of 1/6 molar sodium acetate, the total amount administered being 15 mEq per kg of body weight. Blood specimens were taken under oil at intervals of 15 minutes or longer, and serum CO_2 was determined by the manometric method of van Slyke. Studies on 8 dogs yielded uniform results and showed a rapid rise in CO_2 content. The observations from 5 experiments with similar rates of infusion are given in Table I. Within 15 minutes after starting the sodium acetate infusion the Serum CO_2 showed a prompt elevation and continued to rise during the infusion, leveling off in the afterperiod without further significant change. Infusion of the same