Fermentable Chromogen in Ampuled Inulin and Its Renal Clearance. (20956)

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The resorcinol method of Schreiner(1) for the determination of inulin is simple because it does not entail yeasting. In the past when ampuled inulin was used, this omission seemed justified since the inulin contained only 2% fermentable material(2). However, in recent dog experiments high creatinine-inulin clearance ratios were noted whenever ampuled inulin was measured by this method without yeasting. We have tested 5 lots of inulin which were available and found up to 50% fermentable material to be present. The significance of this finding in renal clearance studies is apparent in the data given below.

Methods. Three or more clearance periods were obtained in each experiment, anesthetized dogs (nembutal) being used. Approximately level plasma concentrations of test substances, inulin 30 mg % and creatinine 20 mg % were maintained by a constant infusion pump. Concomitantly, a saline infusion insured a steady urine flow approximating 3% of the estimated filtration rate. Creatinine was determined in trichloracetic acid filtrates of both plasma and urine(3) and inulin in yeasted and non-yeasted cadmium sulfate filtrates of both plasma and urine. Urine samples were diluted to an expected U/P ratio of one, and in the final calculations, predetermined urine and plasma blanks were subtracted from experimental values. Water recoveries were performed as follows: Ampules of inulin were first warmed at temperatures below 100°C. Buffered inulin (USS inulin*) invariably went into solution quickly. Ampules containing no buffer (WW inulin), usually required about 10 minutes of heating before all crystals went into solution. After cooling, stock solutions of 20 mg % were prepared from each ampule.

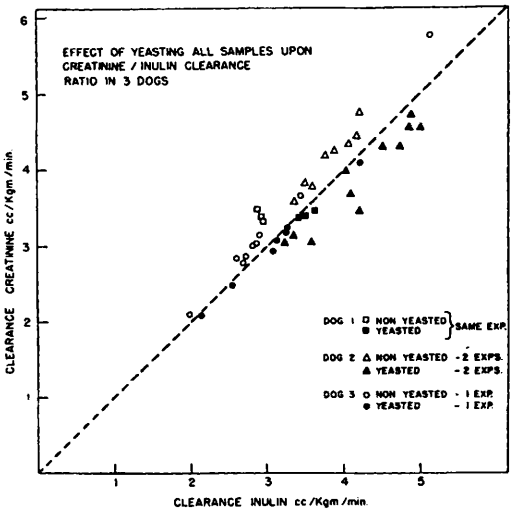

FIG. 1.

Further dilutions were subjected to acid hydrolysis and colored by resorcinol(1) both without yeasting and after yeasting. Yeasting recoveries were carried out by adding 2 ml of 20 mg % inulin to 8 ml of yeast suspension having a measured concentration of yeast cells. The mixture was allowed to stand with occasional shaking for 15 minutes at room temperature after which it was centrifuged for 10 minutes at 2000 g. The yeast blank from fresh Fleischman's yeast, washed 10 to 13 times before use, rarely contained significant amounts of reducing substance.

Results. Data from 7 experiments on dogs shown in Fig. 1 show that treating both plasma and urine samples with 40% yeast suspension raises the inulin clearance and the inulin/creatinine clearance ratio.

The variations among the 2 sets of data in Fig. 1 (open and closed symbols) are attributed to individual differences between animals and to differences in the amount of fermentable chromogen in various lots of inulin.†

* Preparations are "USS" = U. S. Standard Products inulin, Woodworth, Wisc. "WW" = William Warner inulin, Warner-Chilcott Laboratories, New York City.

† Unfortunately, the lot numbers were not recorded for each experiment, but we subsequently have examined each lot that was available to us.

TABLE I. Effect of Yeasting on Renal Clearance Data in a 19.1 kg Dog.

	Collection period			
	0	1	2	3
Urine inulin, mg %				
Not yeasted	2.8	474	413	496
Yeasted	5.5	464	398	480
Plasma inulin, mg %				
Not yeasted	.96	20.8	20.0	20.0
Yeasted	.46	16.6	16.0	15.0
Fermentable chromogen, %				
Urine		2.1	3.6	3.2
Plasma		20.2	20.0	25.0
Urine flow, ml/min.	.82	2.29	2.53	2.09
Clearances, ml/min.				
Inulin, not yeasted	2.4	54.6	54.8	54.4
" , yeasted	9.8	65.8	64.7	69.0
Creatinine		64.9	64.2	66.4
C_{cr}/C_{in}				
Not yeasted		1.19	1.17	1.22
Yeasted		.99	.99	.96

Table I gives the data for 3 urine collection periods in one representative experiment. If it is assumed that the fermentable component is freely filterable, its partial failure to appear in the urine must reflect tubular reabsorption.

The importance of yeast concentration in the analysis for inulin was studied by noting the relationship between the amount of inulin lost and yeast used. This was found to vary with each of the different lots of inulin. Fig. 2 summarizes the results, but no precise quantitative significance should be given to the several curves. Because different ampules agree reasonably closely within a given lot of either brand it is evident that the contaminating material is characteristic of entire lots rather than occasional ampules.

Even the more homogeneous lots showed an increasing loss of inuloid chromogen when the proportion of cells in the yeast suspension was raised from 10 to 40%. A similar phenomenon has been observed with respect to non-fermentable reducing substances occurring in blood(4) and with xylose(5) and has been attributed to partial permeation of these substances into the intracellular water of yeast cells. The curvilinear slope for yeast absorption seen in Fig. 2 could reflect several processes including fermentation of fructose and oligosaccharides, and the action of small amounts of specific inulinase, as well as diffusion.

We have made no attempt to determine the more exact nature of the fermentable material present in commercial preparations. Cotlove (personal communication) has found 26% copper reducing activity (in fructose equivalents) in one lot of the material discussed here (USS lot No. 229A1) and on paper chromatography, fructose, disaccharides and higher oligosaccharides were present in significant amounts. Most available inulin (powdered or ampuled) apparently contains 15 to 40% alkali labile material diffusing faster than the alkali stable material which behaves like a larger molecule. Young and Raisz(6) have compared the simultaneous renal clearances of these 2 fractions in the dog and found them to be equal. Free fructose could conceivably be produced by hydrolysis of inulin within the ampule, during warm storage of ampules containing a slightly acid buffer. We have noted no relationship between fermentable chromogen content and storage time.

Summary. A comparison has been made of the fermentable chromogen content in several lots of 2 commercial brands of ampuled inulin. It is shown that as much as 50% of some currently available ampuled inulin cannot be recovered after mixing with yeast. A lower creatinine/ampuled-inulin clearance ratio has been found in dogs when samples of plasma and urine are treated with yeast prior to analysis. This indicates that when present, fermentable material is probably reab-

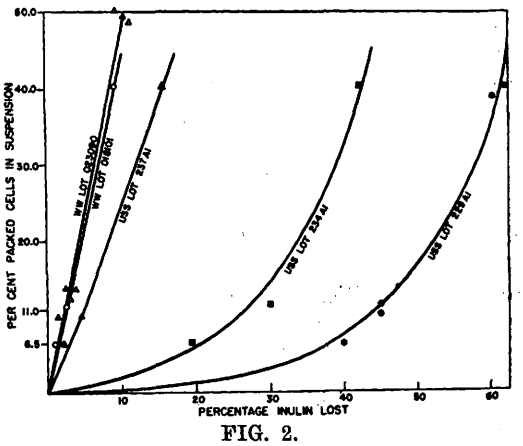

FIG. 2.

sorbed by the renal tubule. Ampuled inulin suspected to contain it should not be used for measuring filtration rate unless all samples are yeasted before analysis.

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Inhibition of Serous Exudation and Tumor Cell Proliferation in Peritoneal Cavities of Mice Given Cortisone.* (20957)

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Previous tests for effects of cortisone on various mouse tumors (including Sarcoma 180) have revealed as a rule no inhibition of their growth(1-3). Moreover, several reports have stated that cortisone treatment facilitated the growth of transplanted tumor cells (4) by modifying host environment, *i.e.*, by "diminishing the awareness of the host"(5) or by inhibiting antibody formation(6). On the other hand, some authors working with different tumors and under different experimental conditions recorded complete(7) or moderate(8-10) inhibition of tumor growth. However, no investigation on this problem has thus far been carried out with mouse tumors growing in a peritoneal exudate and on the serosa. Sarcoma 180 cells can be cultured *in vivo* in this way(11,12), and it occurred to us that these experimental conditions might provide a suitable tool for measuring quantitatively the effect of cortisone both on tumor cells and on their environment. Accordingly, we have inoculated mice i.p. with requisite numbers of Sarcoma 180 cells and treated some of them with cortisone while keeping others as controls. Later, the amount and cellular composition of the exudate in the peritoneal cavities of the experimental and control mice were determined, as was also the amount of tumor implanted on the serosa. The results are reported below.

Material and methods. 1. *Tumor and animal strains.* We have previously described (12) the ability of S-180 to grow as free cells in serial transfers in the peritoneal or pleural exudate of CFW mice. 2. *Inoculation and exploratory abdominal puncture.* Doses of inoculated tumor cells were high enough (10 to 15 millions) to insure takes and to reduce individual variations. Technics of inoculation, of counting tumor cells and of measuring the amount of peritoneal exudate by means of glass capillaries have been described elsewhere(11). 3. *Treatment.* Cortisone and hydrocortisone, both alcohol free and acetate preparations† were used, but since they produced similar effects the results were pooled. All mice received the same total dose of cortisone (5 mg); this was started on the day of implantation and given in one or 2 daily doses either of one mg (concentrated treatment) or of 0.5 mg (fractionated treatment). Control series of treatments were given to mice with peritoneal exudates induced by the i.p. injection of normal tissue cells (ground liver, spleen, lung, and brain) in order to study the effect of cortisone on exudate devoid

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