present. Heating the first filtrate (containing much protein) at P_H of 4.0 does not always produce a coagulum and if not, may increase the potency.

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Precipitation reactions of insulin.

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We here report the results obtained by the addition of various reagents to the aqueous solution of insulin as it is prepared for injection in human cases. Potency tests were made on normal rabbits, the dose given being equivalent to 20 gms. of pancreas except in a few of the earlier experiments where the equivalent of 40 gms. was taken. Only those instances where some positive test was obtained with the same material treated at the same time are reported.

Up to date 29 reagents embracing a wide range in chemical nature, have been tried of which two (formaldehyde and ethyl acetate) gave no precipitate of any kind. Next comes a list of 6 which gave a definitely negative result, *i. e.*, contemporary experiments with the same material gave some positive tests with other reagents. It should here be explained that on the addition of the reagents the precipitate is thrown down by centrifuge taken up in sterile water and injected, the supernatant liquid is dried down, with or without dialysis, depending on the nature of the precipitant, and similarly dissolved and injected. The following 6 gave definite negatives: ether, petroleum ether, toluene, xylene, chloroform and cadmium chloride. Five more are probably destructive to the principle, phosphotungstic, phosphomolybdic and tannic acids, $MgSO_4$ and $NaSO_4$ although the evidence is not yet conclusive. Mechanical difficulties were encountered with phenyl hydrazine, pyrogallic and picric acids, which have eliminated them from our list. Too little has been done with UO_2Ac_2, Zn SO_4 and NaCl to justify any inferences.

Turning now to the positive results we find that absolute ethyl alcohol is unique in being the only reagent encountered so far

that throws down a precipitate from aqueous solution but leaves the insulin in solution. Eight of the nine remaining reagents have given consistent and good positive precipitations. Of these ammonium sulphate is the only inorganic reagent and our results with it have been far from startling, although Shaffer claims that by means of one-half saturation with it insulin is thrown out of solution in the globulin fraction. Acetone, trichloracetic acid and the six alcohols, methyl, isopropyl, n-propyl n-butyl, n-amyl, and n-caprylic have rarely failed to yield a potent precipitate.

In the table all potency values except the first few represent the drop in the blood sugar of the normal rabbit brought about in a period of two hours by the injection of the equivalent of 2 c.c. of the original or 20 gms. pancreas.

TABLE I.

EFFECT OF PRECIPITATION ON POTENCY*

Extract No.	Precipitant 5 vols. added	Original Potency	Potency of precipitate	Potency of filtrate
84-2	Absolute alcohol	—.045	+.017	—.085
84-2	Acetone	—.045	—.034	±.000
84-2	Toluene	—.045	—.003	—.029
84-2	Chloroform	—.045	—.013	—.004
75-3	Absolute alcohol	—.061	—.010	—.070
75-3	Ether	—.061	+.011	—.018
75-3	Chloroform	—.061	—.015	—.001
87-3	Acetone	—.047	—.036	—.009
87-3	Acetone reppt.	—.047	—.049	
101AB	n-Butyl alcohol	—.072	—.075	—.081
101AB	n-Amyl alcohol	—.072	—.082	+.003
105	Trichlor-acetic	—.087	—.069	+.003
106B	n-Butyl alcohol	—.028	—.039	—.003
106B	n-Amyl alcohol	—.028	—.052	—.009
106B	n-Caprylic alcohol	—.028	—.050	
114A	Trichloracetic	—.007	—.046	
114A	Ammonium sulphate[1]	—.007	—.030	
120I	n-Butyl alcohol	—.056	—.057	
120I	n-Propyl alcohol	—.056	—.071	
120I	Iso-Propyl alcohol	—.056	—.041	
120I	Methyl alcohol	—.056	—.056	—.004
120I	Methyl alcohol	—.056	—.033	+.003
119 III	Ammonium sulphate[1]	—.037	—.024	
119 III	Ammonium sulphate[2]	—.037	—.013	
119 III	Magnesium sulphate[1]	—.037	+.015	
119 III	Magnesium sulphate[2]	—.037	no ppt.	
119 III	Sodium chloride[1]	—.037	+.012	
119 III	Sodium chloride[2]	—.037	—.051	
119 III	Sodium sulphate[1]	—.037	.000	
119 III	Sodium sulphate[2]	—.037	+.010	

[1] one-half saturation [2] complete saturation

* These results are chosen at random. They are not to be taken as bases for the conclusions, a complete report at the present time requiring more space than its value would warrant.

There are a few instances of considerable rise, far beyond any experimental error. Although these occur only in those cases where inorganic salts were present and can probably be explained on the grounds that these were not entirely removed, the facts are not without interest. Using $CdCl_2$ in an attempt to remove lecithin, the filtrate of No. 93.2 showed a rise of 339 mg. against an original drop of 96 mg. The precipitate thrown down by $(NH_4)_2SO_4$ in No. 108x showed a rise of 79 mg. against an original drop of 69.

There are other instances where a slight rise was consistently found, but being within the limits of experimental error the results may not be significant. This is noticed especially in the case of the precipitate thrown down by alcohol and in the filtrates, when inorganic reagents are used even though dialyzed and though the evaporated solution shows no microscopic evidence of the crystals of the substance used.

A series of experiments was conducted with acetone as a precipitant with three equivalent portions of varying acidity, one made slightly acid, the second practically neutral and the third slightly alkaline. In those results which gave positive tests there was a very marked gradation first in the potency and second in the nitrogen content. The most acid of the three yields the most potent precipitate and has the lowest nitrogen value.

TABLE II.
EFFECT OF REACTION ON PRECIPITATION BY ACETONE

Extract Number	Precipitant	Reaction or P_H	Original Potency	Potency of ppt.	Nitrogen %
98-2	Acetone	slightly acid	—.090	—.039	—
98-2	"	neutral	—.090	—.039	—
98-2	"	slightly alkaline	—.090	—.023	6.54
99-2A	"	5.8	—.068	—.094 —.071	4.26
99-2A	"	6.7	—.063	—.030 —.023	4.92
99-2A	"	8.8	—.063	—.041 —.053 +.007	6.08
103-1	"	5.7	—.046	—.008 +.004	10.3
103-1	"	6.7	—.046	—.003 +.015	10.2
103-1	"	7.8	—.046	—.028	10.0