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Precipitation of Insulin with Rhodamine-B.

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A few years ago one of the authors in unpublished results showed that rhodamine-B would precipitate pepsin from solution. Since the well known proteolytic ferment precipitating agent, safranine, also precipitates insulin,[1] it was decided to determine if rhodamine-B would precipitate insulin and if so, whether the dye could be removed without inactivation of the insulin. Our experimental results show that insulin is readily precipitated by rhodamine-B at pH 7.2. The complex is soluble in distinctly acid solution, probably with decomposition into its components, for the dye can be practically completely removed by extraction with isoamyl alcohol, leaving the insulin behind in the aqueous layer.

To 1 cc of insulin, Lilly, (40 units per cc) 1 cc of a phosphate buffer solution of pH 7.2 was added, followed by 1 cc of a saturated solution of technical rhodamine-B which was either centrifuged or allowed to stand for several days to remove water insoluble impurities. Almost immediately after addition of the dye a flocculent precipitate separated out. After standing for approximately 10 minutes the precipitate was removed by centrifuging and washed with approximately 3 cc of the buffer solution. The washed precipitate was then dissolved in 1 cc of 0.1N HCl and diluted to 4 cc. Typical blood sugar changes following its subcutaneous injection into rabbits are shown in Table I. Although the number of animals used was not sufficient to give accurate data on the percentage recovery they do show, however, that it is reasonably satisfactory.

In other experiments, after the insulin-rhodamine-B complex was dissolved, the solution was extracted several times with isoamyl alcohol to remove the rhodamine-B. These extractions left a faint pink color in the aqueous layer which we were unable to remove by additional extractions. In order to remove the dissolved isoamyl alcohol the aqueous layer was extracted several times with anhydrous ether. The dissolved ether was removed by allowing the solution to stand at room temperature overnight. The volume was measured and the solution diluted so as to make it theoretically equivalent to 10 units of insulin per cubic centimeter. The blood sugar changes

[1] Jacobs, H. R., and Ricketts, H. T., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 473.

TABLE I.
Effect of the Various Preparations on Blood Glucose Concentration.

Theoretical insulin unitage per kg	Blood glucose per 100 cc, mg			Material used
	Before	After 2 hr	After 4 hr	
1.25	107	47	96	Rhodamine-insulin complex
1.25	121	78	113	" " "
1.5	125	72	65	" " "
1.5	120	75	81	" " "
1.5	125	62	55	" " "
2.0	111	48	86	" " "
2.0	110	62	65	" " "
2.0	109	50	61	" " "
2.0	120	53	Convulsions	" " "
3.0	108	49	Convulsions	Isoamyl alcohol extracted material
3.0	108	43	"	" " " "
3.0	111	60	75	" " " "
3.0	111	60	83	" " " "
3.0	115	47	Convulsions	" " " "
1.5	112	56	75	Insulin, Lilly
2.0	120	64	68	" "
2.0	124	43	Convulsions	" "
2.0	118	84	"	" "

following its subcutaneous administration to rabbits are shown in the table. It will be seen to possess marked insulin activity.

While this work was in progress articles by Lasch and Schönbrunner[2, 3] came to our attention in which it was shown that rhodamine, as well as a few other substances, will protect insulin against digestion by trypsin. These authors have further shown that if insulin is mixed with dyes which will protect it against both tryptic and peptic digestion then its oral administration along with saponin leads to considerable insulin absorption. Experiments along these lines using the insulin rhodamine-B complex are contemplated for the near future.

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2 Lasch, F., and Schönbrunner, E., *Arch. f. Exp. Path. u. Pharmakol.*, 1936, **182**, 452.

3 Lasch, F., and Schönbrunner, E., *Klin. Wochenschr.*, 1938, **17**, 1177.