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Note on the adsorption of insulin by kaolin.

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Considerable progress has been made in the purification of enzymes since Willstätter and his coworkers have perfected the method of adsorption and elution.[1] It occurred to us that these methods might be successfully used in the further purification of insulin. Quantitative studies of the behavior of insulin in adsorption are difficult owing to the necessity of assaying the insulin physiologically. This difficulty, we believe, may now be overcome by the use of the iodometric method of estimation.[2]

This report deals with our studies on the adsorption of insulin by kaolin. The kaolin used in our experiments was a special brand, purified by electrodialysis.[3] The insulin preparation used (Lilly lot 769999, 20 units per mg.) contained 440 units per cc., and had an iodine value of 21.7 cc. 0.01 N iodine in neutral-buffered solution and of 34.5 cc. 0.01 N iodine in alakaline solution. The preservative was removed from the insulin preparation by isoelectric precipitation, because phenols interfere with the iodometric estimation. There were adsorbed 440 units of insulin on varying amounts of kaolin for 15 min. and centrifuged. The supernatant fluid is designated "adsorption rest-solution." The insulin was then removed ("eluted") from the kaolin with 0.01 N NH_3 and centrifuged. The insulin was precipitated from the supernatant fluid (designated "elution" by Willstätter) at pH 5 and centrifuged. The supernatant fluid is designated "elution rest-solution." The table shows our results on adsorption and elution, the percentages of insulin recovered from the solutions corresponding to the different steps in the procedure, and the iodine values from which the percentages of insulin were calculated. The amount and potency of the insulin in the adsorption

[1] Terminology according to Willstätter.

[2] Brand, E., and Sandberg, M., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 313.

[3] Wallstätter, R., and Waldschmidt-Leitz, E., *Z. physiol. Chem.*, 1923, cxxv, 132, p. 180.

INSULIN ADSORPTION BY KAOLIN.

Exper. No.	Adsorption of 440 un. at pH 3		Adsorption Rest-solution			Elution with 10 cc. of 0.01 N NH_3						Total I_2 value recovered.	
						Precipitate at pH 5			Elution Rest-solution				
			cc. 0.01 N I_2		% insulin (I_2 absorbing material.)	cc. 0.01 N I_2		% insulin (I_2 absorbing material.)	cc. 0.01 N I_2		% insulin (I_2 absorbing material.)		
	vol.	kaolin	neutr.	alk.		neutr.	alk.		neutr.	alk.		neutr.	alk.
5	10 cc.	0.2 gm.	14.40	24.50	66*	4.80	7.90	22*	1.50	2.50	5	20.70	34.90
9	30 cc.	0.2 gm.	14.56	22.80	67*	6.70	11.35	31*	0.36	0.72	1.5	21.62	34.87
6a	10 cc.	0.4 gm.	7.20	10.10	33*	11.25	16.75	52*	3.18	6.30	15	21.63	34.40
7	10 cc.	0.6 gm.	1.40	1.80	6.5**	19.90	29.60	92*	0.36	1.35	1.5	21.66	32.75
8	10 cc.	0.8 gm.	1.40	1.80	6.5**	18.10	29.60	83*	1.84	1.68	8.5	21.34	33.08

*Tested physiologically and found active.
**Tested physiologically and found inactive.

rest-solutions and in the precipitates from the elutions has been roughly assayed physiologically as a check for the iodometric estimations.

Our experiments show that relatively large amounts of kaolin are required for the adsorption of insulin. Kaolin apparently is not a very specific adsorbent for insulin and it remains doubtful whether it can be used advantageously in the further purification of insulin preparations. Willstätter's special aluminum hydroxide preparations are sometimes extremely specific and selective adsorbents, depending on the type of preparation and the enzyme. Our preliminary experiments with aluminum hydroxide (preparations B and C according to Willstätter) seem to indicate that it is a more suitable adsorbent for insulin than kaolin.

Further experiments are necessary to establish more fully the behavior of insulin in adsorption and elution. It may be possible that such studies will lead to a practical method for the further purification of insulin.

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Glutathione in blood and its utilization in milk secretion.

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The work reported was originally an attempt to follow free cystine in blood by means of the Folin-Looney[1, 2] method. It was planned, if possible, to repeat with cystine the work done in this laboratory with tryptophane[3, 4] and thus to follow its utilization by the mammary gland in milk secretion, and to study further the changes that may occur in the composition of the blood mixture of amino acids as a result of various changes in diet.

Protein free blood extracts were made as in the amino N de-

[1] Folin, O., and Looney, Joseph M., *J. Biol. Chem.*, 1922, li, 421.

[2] Looney, Joseph M., *J. Biol. Chem.*, 1922, liv, 171.

[3] Cary, C. A., and Meigs, Edward B., *J. Agr. Res.*, 1924, xxix, 603.

[4] Cary, C. A., *Proc. Am. Soc. Biol. Chem.*, Dec., 1925.