

same is true of vaccine virus. The dried material of Chicken Tumor I is rendered inactive by shaking with chloroform while vaccine virus in the dried state withstands such treatment. *Herpes febrilis* in brain tissue resists freezing and desiccation but is destroyed when the dried material containing it is shaken with alcohol, acetone, toluol, or chloroform. None of the agents studied are extracted in their active form from the dried material by the solvents.

From these results it would appear to be impossible to distinguish between the agent of Chicken Tumor I and viruses by desiccation and subsequent treatment with the ordinary organic fat solvents.

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Reaction Between Proteins and Diazotized Aromatic Amines in Neutral Solution.*

MICHAEL HEIDELBERGER AND FORREST E. KENDALL.

From the Laboratories of the Department of Practice of Medicine, Presbyterian Hospital and College of Physicians and Surgeons, New York City.

In a series of 3 challenging papers entitled "Chemospecific Antigens" Klopstock and Selter¹ have raised a number of important questions dealing with the chemical basis of certain immune reactions. While several of their minor points will be dealt with in connection with other work now in progress, the present note concerns the underlying thought and principal conclusion of their work, namely, that no chemical interaction takes place in neutral solutions of diazotized aromatic amines and proteins or lipoids. It is held that such solutions are merely simple mixtures, and that "eine chemisch zu verstehende Substitution des Chemikals im Eiweissmolekül bei der chemospezifischen Komplexantigenbildung keine Rolle spielt." Klopstock and Selter have failed entirely to report chemical control experiments in testing this conclusion, and the following are therefore submitted:

I. Conditions the same as in Paper II, p. 455, showing appearance of chemospecific antigen only after mixtures had stood in the cold 1 to 24 hours.

1% diazotized arsanilic acid, "neutralized to litmus."

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¹ *Z. f. Immunitätsforschung*, 1928, iv, 118, 450; lvii, 174.

2 vols. rabbit serum (K. and S. used beef serum in this experiment, rabbit serum in others).

Dubose Colorimeter* Readings

Immediately after mixing		1 hour in ice-box after mixing	
1 vol. diazo solution, 2 vols. H ₂ O	Diazo-serum mixture	1 diazo, 2 H ₂ O	Diazo-serum mixture now orange in color
	4	6.3	1.1
5 min. later at room temp.	4	6.3	1.1
13 min. later at room temp.	4	0.4	

* Thanks are due Prof. H. T. Clarke for suggesting the greater precision obtainable with the colorimeter.

Since color formation could conceivably have been due to some other serum component than protein, a solution of 3-times recrystallized, dialyzed egg albumen (containing 0.018 g. per cc.) was next used.

II. No difference to the eye immediately after mixing.

Dubose Colorimeter Readings after 1 hour in ice-box

1 vol. diazo solution, 2 H ₂ O	1 diazo solution, 2 egg albumen
2.3	1.7

Since the neutral diazo arsanilic acid solution darkened rapidly, as noted by Klopstock and Selter, making readings after several hours unsatisfactory, diazotized sulfanilic acid was substituted. This darkens comparatively little on standing in the cold, and, in addition, antisera to sulfanilic-azo-proteins were available. Moreover, since "neutrality to litmus" is a vague term an experiment was run at pH 6.8, but since it might be objected that this is toward the alkaline side of "neutral to litmus" and that actual coupling took place for this reason,† there are given below only the results at pH 6.3-6.4, at which blue litmus is faintly reddened.

III. Dialyzed egg albumen solution, 0.022 g. per cc., pH 6.3. 0.75% solution of diazotized sulfanilic acid, pH 6.4.

† This affords the simplest explanation of the greater tendency of Klopstock and Selter's "serum-control preparations" to yield chemospecific antigens. After being subjected to alkali and acid the products were dissolved "bei alkalischer Reaktion." (Paper II. p. 452.)

Klett Colorimeter Readings

A. After 6 min. in ice-water a faint yellow color appeared only in the albumen-diazo solutions.			
10 cc. H ₂ O		10 cc. H ₂ O	
1 cc. diazo solution		10 cc. egg albumen solution	
		2 cc. diazo solution	
1 min. after removal	40		16.2
18 " " "	40		7.0
B. After 2.5 hours in the ice-box the albumen-diazo solutions were bright yellow, the controls almost colorless.			
0.5 min. after removal	40		6.6
6.5 " " "	40		6.1
C. After 23 hours in the ice-box the albumen-diazo solutions were deep yellow, the controls pale yellow.			
	45		7.8

Tubes set up with protein and M/15 phosphate buffer at pH 6.4 instead of water showed approximately twice as much dye formation.

Serum tests with diazo-albumen solution III C (above)

Diazo-albumen Solution	Anti-Egg Albumen Rabbit Serum, 1:1	Anti-Sulfanilic-Azo-Egg Albumen-Dye Rabbit Serum absorbed with Egg Albumen, 1:1	0.5 cc. of sera and dilutions 2 hrs. at 37°, over night in ice-box
1:10		±	Serum and solution controls negative
1:25		±	
1:100		±	
1:200	++	—	
1:500	+++	—	

It is thus evident that true chemical interaction, with resulting dye formation, takes place in neutral mixtures of diazotized aromatic amines and proteins. In neutral solution it is of course impossible to obtain other than mixtures containing much unchanged protein, unchanged diazo compound, and a little protein dye, but if a buffer or if alkali is present to neutralize the acid liberated, the reaction goes in the direction of complete dye formation. Enough colored compound is formed, however, even in faintly acid solution to be observed readily and to be detectable by the specific precipitin test. Landsteiner's conception of the reaction² is therefore in accord with chemical data, while Klopstock and Selter's main thesis in the series of papers under discussion is utterly without experimental basis.

² *J. Exp. Med.*, 1924, xxxix, 631; *Klin. Wchschr.*, 1927, vi, No. 3.