in a manner similar to the response of patients with adrenal hyperplasia and normal individuals. Therefore, it seems clear that Su 4885 inhibits 11β-hydroxylation in adrenal carcinoma as well as in non-neoplastic glands. The difference in response as regards total urinary ketogenic steroids apparently resides in the failure of the neoplastic glands to respond to stimulation with the endogenous ACTH released after Su 4885 administration. Patients with carcinoma of the adrenal usually do not show rises in plasma or urinary steroid levels when ACTH is administered, nor do their urinary steroids diminish following suppression of ACTH with exogenous steroids.

The response to Su 4885 may prove useful as a clinical test for differentiating adrenal carcinoma from hyperplasia(8) but more data must be amassed before generalizations can be made. Analogs of this compound may also assume a role in the chemotherapy of adrenal carcinoma.

Summary. Administration of Su 4885 resulted in inhibition of 11β-hydroxylation within the adrenal cortex in 7 patients with Cushing's syndrome. In 5 patients with adrenal hyperplasia, this resulted in a rise in values of urinary ketogenic steroids whereas in the 2 patients who had adrenal carcinoma no such rise was observed. This difference in the 2 groups of patients is ascribed to the state of adrenal responsiveness to endogenous ACTH.

1. Jailer, J. W., Gold, J. J., Wallace, E. Z., *Am. J. Med.,* 1954, v16, 340.

2. Grumbach, M. M., Bongiovanni, A. M., Eberlein, W. R., van Wyck, J. J., Wilkins, L., *Bull. Johns Hopkins Hosp.,* 1955, v96, 116.

3. Christy, N. P., Wallace, E. Z., Jailer, J. W., *J. Clin. Invest.,* 1955, v34, 899.

4. Laidlaw, J. C., Reddy, W. J., Jenkins, D., Hayder, N. A., Renold, A. E., Thorn, G. W., *New Eng. J. Med.,* 1955, v253, 747.

5. Gallagher, T. F., *Cancer Res.,* 1957, v17, 520.

6. Soffer, L. J., Geller, J., Gabrilove, J. L., *J. Clin. Endocrinol. and Metab.,* 1957, v17, 878.

7. Liddle, G. W., Island, D., Lance, E. M., Harris, A. P., *ibid.,* 1958, v18, 906.

8. Liddle, G. W., Estep, H. L., Kendall, J. W., Williams, W. C., Townes, A. W., *ibid.,* 1959, v19, 875.

9. Sobel, C., Golub, O. J., Henry, R. J., Jacobs, S. L., Basu, G. K., *ibid.,* 1958, v18, 208.

10. Frantz, A. G., Holub, D. A., Jailer, J. W., *J. Clin. Invest.,* 1960, v39, 904.

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In vitro Passive Transfer of Atopic Hypersensitivity. (25794)

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During experiments on allergic histamine release from leukocytes of atopic subjects(1-3), it was shown that histamine release varies in relation to antigen concentration(1). To study the effect of antibody concentration, attempts were made *in vitro* passively to sensitize leukocytes from non-allergic individuals. That this might be done successfully seemed reasonable in view of the fact that normal skin is regularly sensitized by allergic sensitizing antibody. This is the basis of the Prausnitz-Küstner reaction. Also, Humphrey and Jacques(4) demonstrated that normal rabbit platelets will release histamine in presence of homologous rabbit antibody and antigen.

Methods. Heparinized whole blood was obtained from normal skin test negative individuals. Plasma containing sensitizing antibody was procured from subjects highly allergic to ragweed pollen (3-4 plus reaction to skin test with 1/40 ml ragweed extract containing 100 protein N units per ml). The capacity of ragweed antigen to produce histamine release in the whole blood of the donor subjects is shown in Table I. In some experiments donor plasma was diluted with 0.15 M NaCl prior to mixing with normal whole blood. In other experiments, plasma was col-

TABLE I. Histamine Release in Blood of Ragweed Sensitive Subjects Whose Plasma Was Used for Passive Transfer.

	Plasma histamine conc., μg/l after addition to whole blood of				
		Ragweed (final conc., μg protein N/l)			
Subjects	Saline	2	10	20	50
Gor.	0			69	
	0			70	
App.	0	16			
	4			119	
Ben.	6	36	52		25*
Ten.	5		48		
Dow.	15				175
Kas.	7			93	12*

* Less than maximum histamine release occurs in high antigen excess(1).

lected from the allergic subjects' blood after addition of various amounts of ragweed antigen to the whole blood, *i.e.*, after the histamine release had taken place. The pairs of individuals (donor and acceptor) were matched according to major blood groups (but for one exception, see Table II). To 36 ml of heparinized normal whole blood was added 4 ml of sensitive plasma, plasma dilution, or plasma from ragweed-treated sensitive whole blood. The mixtures were incubated at 37°C for 2 hours with occasional mixing. At this point, saline was added to a control tube and ragweed extract to the other, to give a final concentration of 50 μg protein N per liter. After 30 minutes incubation at 37°C the tubes were centrifuged and plasma collected and analyzed for histamine as previously described(1).

Results. In 10 attempts with 6 sensitive plasmas and 7 normal bloods, only 3 pairs of individuals provided systems which demonstrated passive sensitization as determined by histamine release. As a check 2 pairs were retested and reproducibility was established. Table II shows this information and also demonstrates that one sensitive plasma may not sensitize every acceptor exposed to it (*e.g. Gor* plus Chr +; *Gor* plus Wil −). It further shows that cells of one positive acceptor (Gor plus *Chr* +) may not be sensitized by another sensitive plasma (Dow plus *Chr* −). The explanation of this phenomenon is not known.

Nevertheless, in addition to these interesting findings, several other points are worthy of mention. First is the diminution of histamine release from cells sensitized with plasma dilutions (Table II). This is consistent with the view that sensitizing antibody concentration is one of the limiting factors determining amount of histamine released at constant cell and antigen concentration.

TABLE II. Histamine Release from Passively Sensitized Normal Leukocytes.

System		Histamine released,* μg/l of plasma from normal whole blood plus		
Ragweed sensitive plasma (donor)	Normal cell source (acceptor)	Sensitive plasma (untreated)	Sensitive plasma dilution	Plasma from treated sensitive whole blood¶
Gor. (A+)**	Chr. (A−)	68	0 (1:10)	15§
	" "	50		22§
	Wil. (O+)	0		
App. (A+)	Lar. (A−)	53	23 (1: 2)	39†
	" "	54		15§
Ben. (O+)	Wat. (O+)	19	5 (1: 5)	9,† 8,‡ 0‖
Ten. (A+)	Cra. (A+)	2		
Dow. (A−)	Chr. (A−)	0		
	Whe. (A+)	0		
Kas. (A+)	Whi. (A+)	0		

* Ragweed extract added to final mixtures of normal whole blood-sensitive plasmas to give a concentration of 50 μg protein N/l.

† Prior treatment of sensitive whole blood with 2 μg ragweed protein N/l.

‡ *Idem* 10

§ " 20

‖ " 50

¶ Saline controls ranged between 8-15 μg histamine/l. These are normal values for control plasma.

** Blood group notation; A+ is group A, Rh positive, etc.

Second is the finding that plasma obtained from whole blood previously treated with ragweed antigen confers a diminished capacity upon normal cells to release histamine. The larger the quantity of ragweed used for prior treatment, the less histamine release occurred on subsequent sensitization of normal cells with such plasmas (Table II). This observation strongly suggests that in the initial treatment of sensitive whole blood with ragweed antigen that sensitizing antibody is actually used up in some fashion and hence there is less remaining for subsequent sensitization of the normal cells. Thus, less histamine release occurs on later addition of ragweed to treated plasma-normal cell mixtures. Diminution in transferable sensitizing antibody activity could occur in at least 3 different ways: 1) humoral antibody complexing with antigen, thus preventing reaction of cellular antibody and antigen and subsequent histamine release; 2) binding of antibody to cells during the course of histamine release reaction; 3) formation of a product in the release reaction which inhibits histamine release in a fresh reaction system.

Had the interaction of sensitizing antibody, antigen and cells in the sensitive blood caused generation of any stable anaphylatoxin-like substance, then addition of plasma from such a system to normal blood might be expected to cause a release of histamine without further addition of ragweed antigen. The last column of Table II shows that such plasmas added to normal blood without antigen (saline control) do not bring about a significant release of histamine. It appears, therefore, that this type of allergic reaction is not accompanied by anaphylatoxin generation.

Summary. *In vitro* sensitization of non-allergic human leukocytes by plasma from ragweed sensitive subjects was carried out. In 10 attempts with 6 sensitive plasmas and 7 normal bloods, only 3 pairs of individuals provided systems which demonstrated passive sensitization as determined by histamine release in presence of ragweed antigen. This finding is not related to major blood group incompatibility, and is unexplained. Diluted sensitive plasma brings about less histamine release. Plasma obtained from sensitive whole blood previously treated with ragweed antigen confers a diminished capacity upon normal cells to release histamine. There was no evidence that such plasmas possessed anaphylatoxin-like activity.

1. VanArsdel, P. P., Jr., Middleton, E., Jr., Sherman, W. B., Buchwold, H., *J. Allergy,* 1958, v29. 429.

2. Middleton, E., Jr., Sherman, W. B., Fleming, W., VanArsdel, P. P., Jr., *ibid.,* in press.

3. Middleton, E., Jr., and Sherman, W. B., *ibid.,* in press.

4. Humphrey, J. H., Jacques, R., *J. Physiol.,* 1955, v128, 9.

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Method of Concentration of Viral Diagnostic Reagents Using Hydrophilic Agents. (25795)

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A number of methods other than procedures primarily dependent upon high speed centrifugation and lyophilization have been employed for concentration of viral and rickettsial suspensions. Several chemical methods have been described(1,2,3,4,5). Kohn(6) outlined a procedure for concentration of urine using polyvinylpyrrolidone (PVP)* and polyethylene glycol (Carbowax 20-M)† as hy-

* K and K Laboratories, Jamaica, N. Y.

† Carbide and Carbon Chemicals Co., Union Carbide and Carbon Corp., N. Y.

Use of trade names does not imply endorsement by Public Health Service.