panicle, whereas in the diseased plants the spikelets become bound into a cylindrical structure 3–6 in. long, covered with the sclerotioid masses of the fungus (Fig. 1). Individual spikelets and glume of diseased inflorescence were uniformly dwarfed, as compared with healthy ones, and ultimately failed to develop grains. Diseased plants could be detected in early stages before development of inflorescence by the silvery white films of fungus growth covering the upper leaf surface.

The fungus incited systemic infection of plants and developed ephelidial fructifications in exciples which measured 300–500 μ in diameter. The conidiophores are simple or branched, bearing numerous acicular hyaline conidia that measured 13–23 × 1–1.5 μ with a mean of 18 × 1.2 μ. When diseased spikelets were mounted in water, large masses of conidia loosened from the exciple were released into the water.

Ephelis and *Balansia* stages have been reported from India on several grass hosts, but none so far has been reported on *Sorghum halepense*. An *Ephelis* stage on *Sorghum vulgare* was described by Bruner (*1*) from Cuba and this has been shown by Diehl (*2*) to be the conidial stage of *Balansia claviceps* Speg. The ephelidial conidia of *B. claviceps* measure 24–40 × 1–1.5 μ; while those of *Ephelis* on *S. halepense* measure 13–23 × 1–1.5 μ and therefore belong to a separate species. A detailed description of the fungus based on cultural and inoculation studies is being published separately.

References

1. Bruner, S. C. *Cuba Estac. Exptl. agron. Informe.* (1931).
2. Diehl, W. W. Agri. Monogr. No. 4, U. S. Department of Agriculture, 82 (1950).

Manuscript received May 4, 1953.

The Relation of Allergy Reagins to Electrophoretic Components of Serum[1]

Dan H. Campbell, John R. Cann,[2] Townsend B. Friedman,[3] and Raymond Brown[4]

Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena

Several years ago we published a preliminary report on the fractionation of serum from egg-sensitive patients by the electrophoresis-convection method (*1*). These preliminary data indicated that circulating antibody capable of giving Prausnitz-Kustner reactions might occur in serum components other than gamma globulin. Since then, these observations have been confirmed by other investigators (*2, 3*), as well as our subsequent studies which are reported here. All fractions were tested for their ability to sensitize normal skin passively and to combine specifically with ovalbumin as described by Miller and Campbell (*4*).

Methods

Fractionation of serum. The electrophoresis-convection fractionation of serum was carried out in essentially the same manner as described by Cann, Brown, and Kirkwood (*5*). Two 70-ml samples of whole serum were each diluted to 120 ml and run simultaneously in two separate units. Identical fractions were combined in order to supply sufficient material for the different analyses. Fractionation was carried out in phosphate buffer at pH 7.0 and an ionic strength of 0.1. The field strength was about 1.5 v/cm and the time of each run was about 48 hr. The material present in the top portion of the cell after the first run was labeled T-1 and set aside for testing. The material in the bottom portion of the cell was refractionated and the resulting fractions designated as T-2 and B-2. The B-2 fraction was further separated into albumin and globulin fractions by one-half saturation of the solution with ammonium sulfate at pH 7.8.

Characterization of fractions. The electrophoretic components of each fraction were determined in the usual manner using barbital buffer at pH 8.4. Mobilities were calculated in accordance with the suggestions of Longsworth and MacInnes (*6*). The apparent relative concentrations of electrophoretic components were determined by finding the ratio of the component area in descending patterns to the total area, exclusive of the ε boundary. The areas were measured on projected tracings of the descending patterns with a planimeter. Although both gamma and beta components usually resolved into two fractions (γ_1, γ_2, β_1, β_2), this detail did not seem to be of sufficient significance to record this separation in Table 1. The usual designations of albumin, α_1, α_2, β, and γ, were given to components having mobilities in the range of 6.0, 5.0, 4.0, 3.0, and 1.0, respectively.

Serological properties. All fractions were tested for their specific affinity for ovalbumin by methods described by Miller and Campbell (*4*). This value is expressed as the percent of the fraction that apparently combined with ovalbumin and was brought down as additional protein nitrogen upon addition of a standard amount of rabbit antiovalbumin serum. The amount of the respective fraction which was added to the standard precipitating system was always 7.0 mg. Protein analysis of precipitates was made in the usual manner using the Nessler reaction as described by Lanni and Campbell (*7*).

Analysis for reaginic activity. The protein concentration of various fractions was determined and then adjusted to a value of 7.0 mg per ml and sterilized by filtration. Varying dilutions in 0.1-ml amounts were then injected intradermally into normal volunteers. Forty-eight hours later the prepared sites as well as control sites were tested with intradermal injections of antigen. The reaginic activity was expressed as the limiting dilution of the 0.7 percent protein solution which produced a definite passive transfer reaction.

[1] This work was supported in part by the Public Health Service and in part by the Rockefeller Foundation.

[2] Present address: University of Colorado, Department of Medicine, Denver.

[3] 104 South Michigan Ave., Chicago, Ill.

[4] Lederle Laboratories, Pearl River, N. Y.

TABLE 1. Some properties of reaginic serum protein fractions. Distribution of electrophoretic components percentage of fraction).

Fraction	Albumin	α_1	α_2	β	γ	Percent SAP	PK titer
			F-1 *Egg sensitivity only*				
Serum	65	6	6	9	14	0.6	Undiluted
T-1	8	0	0	12	80	1.8	"
T-2	12	0	8	20	60	14.2	1: 10
BG-2	1	26	29	43	1	27.0	1: 160
BA-2	85	14	0	8	0	0.0	Negative
F-2	*Multiple sensitivity including egg. Ragweed and egg antigens gave the same test reaction*						
Serum	65	2	12	10	11	3.0	1: 10
T-1	8	0	0	24	68	0.0	Undiluted
T-2	10	0	8	29	53	5.3	1: 20
BG-2	18	10	52	11	9	2.7	1: 20
BA-2	84	8	4	4	0	0.0	Undiluted
			M-1 *Egg sensitivity only*				
Serum	61	5	8	14	12	0.0	Undiluted
T-1	7	0	4	16	73	0.0	Negative
T-2	11	0	4	28	62	0.0	"
BG-2	27	7	47	15	4	0.0	1: 20
BA-2	88	6	2	4	0	0.0	Negative
			S-1 *Ragweed sensitivity only*				
Serum	66	3	11	9	11	0.0	Undiluted
T-1	0	0	5	10	85	0.0	Negative
T-2	0	0	5	18	77	0.0	"
T-3	4	2	7	20	68	0.0	"
BG-3	34	9	38	16	3	0.8	1: 40
BA-3	83	8	8	0	0	0.0	Undiluted
		F-3	*Multiple sensitivity, but no egg. Tested with cottonseed*				
Serum	65	5	12	8	10	0.0	1: 10
T-1	7	0	0	13	80	0.0	1: 10
T-2	13	4	8	14	61	0.0	1: 20
BG-2	18	10	52	11	9	0.0	1: 20
BA-2	84	8	4	4	0	0.0	Undiluted
		F-4	*Multiple sensitivity but no egg. Tested with ragweed*				
Serum	59	6	10	14	11	0.0	1: 10
T-1	7	0	0	16	77	0.0	1: 10
T-2	14	0	11	21	54	0.0	1: 40
BG-2	11	7	58	17	7	0.0	1: 80
BA-2	84	8	3	5	0	0.0	Undiluted
			F-5 *Ragweed sensitivity only*				
Serum	49	6	9	21	15	0.0	1: 10
T-1	0	0	0	23	77	0.0	Negative
T-2	0	0	0	26	74	0.0	Undiluted
BG-2	8	7	38	34	13	0.0	1: 20
BA-2	81	11	0	8	0	0.0	Undiluted

Note:
SAP = Specific absorbable protein: The percent of the fraction (7.0 mg) which combined with ovalbumin and was precipitated upon addition of standardized rabbit antiovalbumin serum.
PK titer = based on limiting dilution of 0.1 ml of an 0.7% solution.
T = top. **B** = bottom. **G** = globulin. **A** = albumin.

The PK titer given in Table 1 represents an average value obtained by tests on four different individuals.

RESULTS

The pertinent experimental data are given in Table 1. The nature of tests involved does not afford a precise quantitative evaluation of the data presented. However, it is clearly evident that reagins are not characteristically associated with the gamma globulin component of serum. In most instances analytical data are not precise enough to make any definite conclusions as to what component or components contain

activity. However, in the case of serum S-1 it would appear as though the activity resided almost entirely in the α_2 component. It was also of interest that this preparation contained some material that combined with ovalbumin although the patient failed to show any clinical history of egg sensitivity. Some of the fractions were tested for diphtheria antitoxin activity, typhoid agglutinins, and blood group specific antibodies. The antitoxin and typhoid agglutinins were always present when gamma globulin predominated and the blood group agglutinins were always strongest in the T-2 fractions, as would be expected from previous work. Of the four patients not sensitive to egg white, only one produced a serum fraction which combined with ovalbumin (the BG-2 fraction of S-1 serum). All the M-1 serum fractions failed to combine with ovalbumin, but it was later found that this individual was sensitive only to the ovomucin component of egg white. Although some of the apparent discrepancies in the relation of PK titers to the relative concentration of serum components may be the result of error inherent in the method of titration, there is also a possibility that inhibiting substances may occur in serum. Since these serums all came from individuals who had no previous history of treatment, one would not expect to find classical "neutralizing" antibodies to be present.

References

1. CAMPBELL, D. H., *et al. J. Allergy* **21**, 519 (1950).
2. COOKE, R. A., *et al. Ibid.* **22**, 211 (1951).
3. LOVELESS, MARY H., and CANN, J. R. *Science* **117**, 105 (1953).
4. MILLER, H., and CAMPBELL, D. H. *Ann. Allergy* **5**, 236 (1947).
5. CANN, J. R., BROWN, R. A., and KIRKWOOD, J. G. *J. Am. Chem. Soc.* **71**, 1609 (1949).
6. LONGSWORTH, L. G., and MACINNES, A. *Ibid.* **62**, 705 (1940).
7. LANNI, F., and CAMPBELL, D. H. *Stanford Med. Bull.* **6**, 97 (1948).

Manuscript received November 20, 1953.

The Effect of Carbonic Anhydrase Inhibition on the Composition of Urine and Plasma of the Alligator

Thomas Hernandez and Roland A. Coulson

Department of Biochemistry, Louisiana State University School of Medicine, New Orleans

The administration of a potent carbonic anhydrase inhibitor (6063 or Diamox 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide)[1] causes a marked increase in excretion of Na, K, water, and bicarbonate, a decrease in titratable acidity, and an increase in urine pH in the dog, man, rat, and freshwater catfish (*1–3*). According to modern theory, carbonic anhydrase seems to be essential in mammals for acid secretion by the renal tubules, the H ions competing with Na and K for excretion (*4*). Carbonic anhydrase enables these animals to produce an acid urine and so conserve base.

The electrolytes normally present in the plasma of the alligator (*A. mississippiensis*) (*5*) occur in approximately the same concentrations as those of the human yet the composition of alligator urine is quite dissimilar to that of mammals. Although the major share of the urinary nitrogen is excreted in the form of uric acid (*6*), enough ammonia is produced to maintain the urinary level at about 70 meq/l. In well-hydrated fasting alligators this ammonia is normally excreted with an almost equivalent amount of bicarbonate which is presumably produced through the influence of carbonic anhydrase in the nephron. Of the total osmotic pressure of the urine, which never exceeds that of the plasma, about two-thirds is due to the NH_4 and HCO_3 ions. As the alligator normally produces an alkaline urine (pH 7.80) due to the large amounts of NH_4 and HCO_3 ions, the proof of the existence of a base conservation mechanism in this animal is difficult to obtain.

It was felt that the injection of a carbonic anhydrase inhibitor into the alligator should change his urinary excretion pattern considerably. If carbonic anhydrase is necessary, directly or indirectly, for the production of both NH_4 and HCO_3 ions then the inhibition of carbonic anhydrase would lead to a great decrease in both these components in the urine. On the other hand, if carbonic anhydrase inhibition prevents the formation of bicarbonate in the tubule without affecting ammonia production the ammonia would then be excreted with a different anion.

In a series of experiments several alligators with an average weight of about 1.5 kg were placed in a tank of water for 24 hr to hydrate them. Some were then removed from the tank, injected with 50 mg of 6063 per kilo, and kept dry under the same condition as the controls for the next 4 days. Urine was obtained by catheterization at frequent intervals and was analyzed immediately or quick-frozen until the analyses were done. Sodium and potassium were determined by flame photometry, chlorides by a modification of the method of Schales and Schales (*7*), ammonia by the method of Conway (*8*), CO_2 content by the Van Slyke manometric method, and phosphorus by the method of Kuttner and Cohen (*9*). A Beckman pH meter with the glass electrode designed for blood analyses was used for all pH determinations. Figure 1 shows the effect of 6063 on the experimental alligators compared with controls during the first 24 hr.

It is apparent that carbonic anhydrase inhibition leads to a great decrease in CO_2 excretion without effecting a corresponding reduction in urinary ammonia. The chloride ion has been substituted almost quantitatively for the bicarbonate. This exchange, which is almost the exact reverse of that found in mammalian experiments, results in a decreased urinary pH and a mild alkalemia. By the 3rd day there was a rise of about 7 meq/l in plasma bicarbonate and a very slight and possibly insignificant fall in plasma chloride. No change in the other plasma electrolytes occurred.

[1] We wish to thank Lederle Laboratories Division of the American Cyanamid Company for a generous supply of Diamox.