needles of the adduct are stable in air for short periods of time, but the odor of the amine soon became appreciable above the solid. In warm sunlight the decomposition is rapid and a brown gum is produced.

In attempts to purify the brown, partly decomposed crystals, vacuum sublimation at 70–80° and 10^{-3} mm. was employed. Under these experimental conditions only small amounts of sublimate were observed. The residue consisted of an apparently polymerized material which no longer possessed the solubility characteristics of the addition compound.

When $(C_4H_9)_3N:BF_3$ was dissolved in anhydrous methanol and an excess of a saturated methanol solution of anhydrous ammonia was added, a white crystalline solid was produced. This material could be recrystallized from chloroform and yielded crystals melting at 161–163° (literature value for the melting point of $H_3N:BF_3$, 163°[2]). This observation is indicative of the greater acid strength of boron trifluoride with respect to ammonia than with respect to tri-*n*-butylamine.

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(2) A. W. Laubengayer and G. F. Condike, THIS JOURNAL, **70**, 2274 (1948).

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COMMUNICATIONS TO THE EDITOR

COPROGEN, A NEW GROWTH FACTOR FOR COPROPHILIC FUNGI

Sir:

Members of the genus *Pilobolus* are strictly dung inhabiting fungi. Several investigators[1,2] who have studied this genus in culture have found it necessary to use dung or a dung extract as a necessary component of their culture medium.

On a basal medium adjusted to *p*H 7.6 and containing the following amounts of ingredients per 500 ml.: acid hydrolyzed casein 25 ml.,[3] L-cystine 50 mg., DL-tryptophan 100 mg., uracil 5 mg., thymine 5 mg., adenine 5 mg., guanine 5 mg., xanthine 5 mg., thiamine 0.5 mg., pyridoxine 0.1 mg., calcium pantothenate 0.1 mg., riboflavin 0.2 mg., nicotinic acid 0.5 mg., choline 0.5 mg., inositol 0.2 mg., *p*-aminobenzoic acid 0.2 mg., pteroylglutamic acid 12 μg, biotin 1.2 μg, vitamin B_{12} 2.4 μg, and inorganic salts A and B,[4] the dung extracts could be replaced by the fermentation liquors of a number of species of bacteria and fungi.

From such source materials a brick red crystalline biologically active compound has been isolated. The isolation was effected by solvent extraction, adsorption on florisil and partition chromatography on a Filter-cel column. After several crystallizations from ethanol, the compound evidenced no sharp melting point but darkened and decomposed from 205°. Elemental analyses indicated the following percentage composition: C, 50.96; H, 6.88; N, 10.26; Fe, 6.61. The compound has a broad ultraviolet absorption maximum at 440 mμ in 50% ethanol, $E^{1\%}_{1\,cm.} = 36.6$.

The name "Coprogen" is proposed for this new compound.

The assays were carried out in 125-ml. erlenmeyer flasks containing 20 ml. of medium. The inoculum consisted of uniform amounts of actively growing mycelium in dung agar blocks. After incubation at 25° for five days the pads were harvested by freeing the mycelium from the small agar block and then drying the mycelium pad at 105° for 16 hours. Weights of the pads were used as a measure of growth.

The addition of 5 millimicrograms of Coprogen per ml. of medium allowed for growth and fruiting of *P. kleinii* with no loss of vigor through ten serial transfers. The compound was equally effective in all the species of *Pilobolus* studied.

Page[5] has recently reported that hemin can partially replace the usual dung extracts in the nutrition of *Pilobolus;* however, the growth was much less vigorous. Under our experimental conditions hemin exhibited only one one-thousandth the growth promoting activity of Coprogen on a weight basis.

(1) A. H. R. Buller, "Researches on Fungi," Vol. VI, Longmans' Green and Co., New York, N. Y., 1934.

(2) E. A. Bessey, *Michigan Academy of Sciences, Arts and Letters*, **32**, 15 (1948).

(3) 1 ml. is equivalent to 100 mg. of casein.

(4) E. E. Snell and F. M. Strong, *Ind. Eng. Chem.*, **11**, 346 (1939).

(5) R. M. Page, paper presented at the Am. Inst. of Biol. Sci. Meetings, U. of Minn., Sept. 1951.

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THE VALENCE OF PRECIPITATING ANTIBODIES

Sir:

Following the studies of Pappenheimer, Lundgren and Williams,[1] a number of investigators[2,3,4] have recently applied the techniques of electrophoretic and sedimentation analysis to the problem of antibody valence.

These studies and ours indicate the dependence of the molecular composition of antigen–antibody complexes upon the relative amount of antigen

(1) A. M. Pappenheimer, Jr., H. P. Lundgren and J. W. Williams, *J. Exp. Med.*, **71**, 247 (1940).

(2) J. R. Marrack and H. Hoch, *Brit. J. Exp. Path.*, **32**, 212 (1951).

(3) S. J. Singer and D. H. Campbell, THIS JOURNAL, **73**, 3543 (1951), "Communication."

(4) J. L. Oncley and D. Gitlin, *J. Phys. Chem.*, **56**, 85 (1952).

present in the system. If it is assumed that each antigen molecule in the complexes is bound directly to some antibody molecule, then the average mole ratio of antigen to antibody in the complexes, $\overline{(Ag/Ab)}_{MC}$, gives directly a measure of the average number of antibody sites which have reacted. The limiting value of this ratio as the relative amount of antigen becomes infinite gives on the average the maximum number of antibody sites which can react; this is the average value of antibody valence. For this reason we have been especially interested in determining $\overline{(Ag/Ab)}_{MC}$ in the region of very large antigen excess. It is these data that we now wish to communicate.

For the two systems, crystalline bovine serum albumin-rabbit antibody and crystalline human serum albumin-horse antibody, the specific precipitate was formed in the equivalence zone and washed with buffered saline. The precipitate was then dissolved in excess antigen and the solution brought to known volume. Solutions of widely varying ratios of total antigen to total antibody were prepared in most cases by keeping the total antigen approximately constant and varying the antibody content.

The amount of total antigen and total antibody in a given solution was determined by nitrogen analysis, using the micro-Kjeldahl method. The free antigen content was obtained by electrophoretic analysis, using a phosphate buffer containing 0.15 M NaCl (μ = 0.17, pH = 6.9). Measurement of the relative area under the free antigen peak of the ascending pattern gave the per cent. of free antigen. In most cases resolution was complete. The antigen bound in the complexes was obtained as the difference of total antigen and free antigen. The bound antigen divided by the total antibody, multiplied by the molecular weight factor, 2.3, gave the average mole ratio $\overline{(Ag/Ab)}_{MC}$. The choice of this factor is somewhat arbitrary because the *number* average molecular weight of the antibody is not precisely known.

The results obtained are summarized in the table. Two distinct differences are evident between the two systems: (1) the relative amount of antigen required to saturate the reactive sites of rabbit antibody is significantly greater, and (2) the limiting value of $\overline{(Ag/Ab)}_{MC}$ for the horse system seems to be clearly two; whereas, it would seem that a limiting value has not been reached in the rabbit system. Also, the highest values obtained in the latter are well above two. However, because of both theoretical and experimental difficulties encountered in the region of high antigen excess, these values and their significance must be accepted with reservation.

ANTIGEN–ANTIBODY INTERACTIONS

Expt.	Total protein N	Total Ag N	Ab N	Free Ag, %	Free Ag N	Bound Ag N	Total Ag N / Total Ab N	$\overline{(Ag/Ab)}_{MC}$
System A: Rabbit antiBSA-BSA								
6S-3	49.3	31.9	17.4	44.0	21.7	10.2	1.83	1.3_5
5S-3	42.5	31.1	11.4	56.5	24.0	7.1	2.73	1.4_5
8S-3	41.2	31.6	9.6	60.7	25.0	6.6	3.29	1.5_8
7S-3	38.5	31.2	7.3	67.5	26.0	5.2	4.27	1.6_4
2'S-3	36.6	31.0	5.6	73.4	26.9	4.1	5.54	1.6_8
VII	33.3	30.6	2.7	84.7	28.2	2.4	11.3	2.0_4
15S-3	18.5	17.6	0.9	89.9	16.6	1.0	19.6	2.5_5
21	40.2	38.6	1.6	91.5	36.8	1.8	24.1	2.5_9
13S-3	45.1	43.4	1.7	91.7	41.4	2.0	25.5	2.7_1
14S-3	44.5	42.7	1.8	91.0	40.5	2.2	23.7	2.8_1
System B: Equine antiHSA-HSA								
15	43.0	26.8	16.2	35.9	15.4	11.4	1.65	1.6_2
1	52.3	36.1	16.2	44.7	23.4	12.7	2.23	1.8_0
10	103	72.6	30.4	48.4	49.8	22.8	2.39	1.7_3
14	42.4	32.3	10.1	56.9	24.1	8.2	3.20	1.8_7
II	15.4	12.3	3.1	62.7	9.7	2.6	3.97	1.9_3
III	29.0	27.3	1.7	89.1	25.8	1.5	16.1	2.0_3
19	40.6	38.8	1.8	91.7	37.2	1.6	21.6	2.0_4

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