ings and those of Stacey(3) showing that tryptamine is relatively little concentrated by platelets suggest strongly that the structural specificity is largely determined by the hydroxyl group at position 5 of the indole ring.

Summary. Monase indole-3(2-aminobutyl) acetate, a monoamine oxidase inhibitor, and 8 other structurally related compounds with different substitutions on the indole ring and amine side chain inhibited 5-HT uptake by platelets, degree of inhibition being directly related to concentration of inhibitor without particular relation to structure. C^{14}-Monase which differs from 5-HT by the absence of a hydroxyl group in position 5 of the indole ring and by the presence of a different side chain, was concentrated only slightly by platelets. It was concluded that the structural specificity for 5-HT uptake is largely determined by the hydroxyl group at position 5 of the indole ring.

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Hemagglutinins for DNA in Tuberculosis and Histoplasmosis.* (31234)

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Previous reports(1) of auto-tissue substances in tuberculosis have indicated that the basic disease process may be complicated by a secondary immunological response to unknown tissue antigens. This investigation shows that sera from rabbits experimentally infected with tuberculosis and sera from humans with tuberculosis and histoplasmosis contain antibodies, demonstrable by hemagglutination, to a known auto-antigen, DNA.

Sera from 14 of 16 rabbits experimentally infected with bovine tubercle bacilli demonstrated antibodies at some stage of infection. Similar tests were performed on the sera from humans with either tuberculosis or histoplasmosis.

Materials and methods. Serology. The bisdiazotized benzidine (BDB) indirect hemagglutination test described by Bigley *et al*(2), was used. The sensitivity of this test in detection of anti-DNA in SLE sera was found equally as effective as the bentonite method of Bozicevich(3).

Phosphate buffer (pH 7.3) was used throughout as both solvent and diluent. BDB was prepared according to the method of Gordon *et al*(4). The antigen was prepared by coupling thermally denatured DNA to human group O Rh positive erythrocytes with BDB. DNA (Nutritional Biochemicals Corp.) was denatured by boiling a solution for 10 minutes in a water bath, followed by immediate cooling in an ice bath(5).

One-tenth ml of washed, packed red cells was added to 10.0 ml of phosphate buffer containing 1000 μg of denatured DNA; 0.5 ml of 1:16 BDB was added and the reaction was allowed to proceed for 10 minutes at room temperature accompanied by frequent shaking of the mixture. After centrifugation, the supernate was discarded, and the treated erythrocytes were washed with 1:100 serum-phosphate buffer solution (1.0 ml normal serum + 99.0 ml buffer), and the sensitized red cells were prepared as a 2% suspension in the serum buffer.

Serum samples in 0.7 ml amounts were diluted 1:2, inactivated at 56°C for 30 minutes, and absorbed with 0.4 ml of washed, packed, normal human group O Rh positive red cells for 30 minutes at 37°C. Serial dilu-

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tions in 0.5 ml aliquots were prepared, 0.1 ml of the antigen suspension was added to each tube of the serial dilutions, and the tubes were incubated for 30 minutes at 37°C. After centrifugation for 30 seconds in an Adams Sero-Fuge, the tubes were examined for gross evidence of agglutination of the antigen-coupled red cells. A titer of 1:8, or greater, was considered significant.

Normal rabbit serum and rabbit serum containing antibody to thermally denatured DNA [prepared by the method of Plescia *et al*(6)] served as controls throughout.

Inhibitions. DNAase (amorphous; 70,000 Dornase units per mg) from Nutritional Biochemicals Corp., trypsin 1:250 from Difco Laboratories, and chymotrypsin from Armour Laboratories were the enzymes employed. Prior to the coupling procedure, enzymatic digestion of the DNA was carried out at 37°C for 1 hour. This was accomplished by mixing DNAase with substrate in a ratio of 1:10 while the ratio for reacting the proteolytic enzymes with the DNA was 1:5.

Nucleosides, heat denatured DNA, an enzymatic digest of DNA, sRNA, and d-2 deoxyribose were employed as inhibitors of the hemagglutination reaction in 500 μg amounts. One-tenth ml quantities of inactivated, absorbed sera were reacted with the inhibitors at 37°C for 40 minutes. Then 0.05 ml of the 2% antigen suspension was added to each tube. The tubes were incubated at 37°C for 30 minutes, centrifuged, and observed for hemagglutination.

Experimental tuberculosis. Adult rabbits weighing 4 to 6 lb each were injected intravenously with 0.2 mg of viable *Mycobacterium tuberculosis* var. *bovis* (Trudeau 4228-4). The organism had been grown on Lowenstein's medium, harvested in weighing bottles, and diluted with unbuffered saline to the desired inoculum size per ml. Prior to infection, the animals were bled by cardiac puncture, and the recovered sera were stored in the frozen state and subsequently served as reference baseline sera.

The rabbits were bled by cardiac puncture at bi-weekly intervals for a period of 12 weeks post-infection, and the stored sera then tested as described above.

TABLE I. Summary of Anti-DNA Reactivity of Tuberculous Rabbits.

	BDB hemagglutination titers*						
	Weeks post-infection						
Rabbit No.	0	2	4	6	8	10	12
1	—	—	—	16	—	16	—
2	—	—	4	8	8	—	—
3	—	—	—	32	16	4	32
4	—	—	—	—	16	4	—
5	—	—	—	—	8	16	8
6	—	—	—	4	16	—	X
7	—	—	—	—	—	8	—
8	—	—	—	8	128	8	8
9	—	—	—	—	—	—	—
10	—	—	4	8	—	—	4
11	—	—	—	8	—	4	—
12	—	—	—	—	32	8	X
13	—	—	—	32	4	8	—
14	—	—	—	—	—	—	4
15	—	—	—	4	2	32	4
16	—	—	4	16	4	—	—

* Titer expressed as reciprocal of serum dilution.

Experimental results. Bi-weekly samples of the sera of 16 rabbits intravenously infected with *M. bovis*, which became tuberculin positive (0.1 ml P.P.D., 2nd test strength intradermally), showed typical symptoms of tuberculosis (weight loss, respiratory distress, etc.), and survived at least 10 weeks were tested for hemagglutinins to DNA. Only titers greater than 1:8 were considered significant. The results are summarized in Table I. The sera of 14 rabbits, at some stage of infection were positive for anti-DNA. Generally the antibodies appeared 4-6 weeks post-infection. Maximum reactivity for DNA-coupled red cells was present in the sera of tuberculous rabbits by 6-8 weeks post-infection. The titers were definite and repeatable, and ranged to 1:32 with an occasional titer of 1:64 or even 1:128.

Specificity of the anti-DNA was established by the inhibition studies. The hemagglutinins in these sera were not demonstrable for erythrocytes coupled with DNA previously treated with DNAase, while erythrocytes coupled with DNA previously treated with trypsin or chymotrypsin were still agglutinable.

Both heat denatured DNA and an enzymatic digest of DNA completely inhibited the hemagglutination reactions. Deoxycytidine, thymidine, and sRNA partially inhibited these reactions.

TABLE II. Summary of Anti-DNA Reactivity of All Sera Examined by BDB Hemagglutination.

Type of serum	No. tested	DNA positive	% DNA positive
Rabbit tuberculosis*	16	14	87.5
Human "	62	17	29.0
" histoplasmosis	30	9	30.0
Normal rabbit	35	1	2.9
" human	74	3	4.1

* Rabbits considered positive if they were positive in at least 1 of 6 serum samples.

Hemagglutinins for DNA were also demonstrable in the sera of 17 of a total of 62 (29%) tuberculous humans. Similarly, 9 of 30 (30%) serum samples from individuals with histoplasmosis contained antibody to DNA. An attempt was made to assess the relationship between DNA hemagglutination and histoplasmin reactivity, with the latter measured by both complement-fixation and collodion agglutination procedures. No correlation was observed.

The data presented in Table II represent the overall rates of anti-DNA reactivity of the sera examined. The percentage of positive sera among the tuberculous rabbits is much higher than in sera of the tuberculosis or histoplasmosis patients, because 6 serum samples, drawn at bi-weekly intervals, were examined for each rabbit, whereas only one serum sample from each human was examined. Reference to Table I shows that at any one time, the highest rate of positive reactors among the rabbits was 50% (6th week).

Discussion. Since the efficiency of the BDB indirect hemagglutination test has not been previously reported as a measure of anti-DNA, several procedures were employed to verify its efficacy in this regard. Rabbits were immunized with a complex of heat-denatured DNA and methylated bovine serum albumin according to the method of Plescia *et al*(6), and the titers of sera obtained from these rabbits were on the order of 1:128 for anti-DNA when measured by the BDB test. Compared with the bentonite test, this test appeared equally as effective. Furthermore, inhibition studies confirmed that the antibodies were specific for DNA. Specificity appears to reside at the nucleoside or nucleotide level, particularly toward deoxycytidine and thymidine. Inhibition by an enzymatic digest of DNA may be accounted for by the breakdown of DNA into its component nucleotides. Partial inhibition by sRNA is probably attributable to its possession of bases common to DNA.

It was found that most of the experimentally infected tuberculous rabbits produced anti-DNA at some stage of the infection, and interestingly, after a maximum titer was reached at approximately 8 weeks post-infection, the titers declined or even disappeared. Several possible explanations exist; (1) sufficient circulating antibody to be detected by the BDB technique no longer exists, possibly because these antibodies are removed from the circulation by adsorption to DNA antigen made available by tissue destruction in the progressive disease, or, (2) these antibodies are no longer being produced as a result of an acquired tolerance or immune paralysis produced by excess antigen with late stages of the disease.

According to Seligman *et al*(7), the sera of 28% of tuberculous humans receiving prolonged drug therapy contained antinuclear factor demonstrable by immunofluorescence. This was attributed to an isoniazed-induced L.E.-like syndrome. Others(8,9) have also reported observation of drug-induced SLE-like syndromes.

The findings reported here that 29% of sera of individuals with tuberculosis contain antibody to DNA do not refute the possibility that the occurrence of anti-DNA or antinuclear factors in tuberculosis is not a drug-induced phenomenon, since all of the tuberculosis patients who were tested had been treated with antituberculosis drugs. However, it must be noted that development of anti-DNA was a natural occurrence in experimentally-infected tuberculosis rabbits, and furthermore, most of the histoplasmosis sera were obtained from diagnostic samples drawn prior to initiation of therapeutic measure.

Since essentially the same percentage (30%) of human tuberculous and histoplasmic sera contained hemagglutinins to DNA, it would be interesting to examine serum samples from a variety of other chronic infectious diseases to determine if the presence of

this antibody constitutes a general immunological principle. Equally pertinent for investigation would be the role of anti-DNA in these diseases. However, in systemic lupus erythematosus sera, a variety of anti-nuclear antibodies have been detected, yet no proof has substantiated their role in contributing to the pathology associated with this disease (10).

From the foregoing, and the results described herein, it is suggested that the presence of antibodies of the general class of autoantibodies warrants study as to their significance in chronic diseases of obvious microbial etiology.

Summary. BDB indirect hemagglutination was determined to be an effective measure of antibody to thermally denatured DNA. It was found equally as effective as bentonite agglutination in detecting anti-DNA in SLE sera. Antibodies to thermally denatured DNA were detected in the sera of experimentally-infected tuberculous rabbits. The antibodies appeared 4-6 weeks post-infection, reached a maximum titer at approximately 8 weeks, and declined thereafter. Similar anti-DNA antibodies were also detected in the sera from patients with tuberculosis or histoplasmosis.

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Normal human sera were obtained from the University Hospital Blood Bank, Columbus, Ohio. Only those serum samples from acceptable blood donors who demonstrated no overt signs of disease were employed.

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Mesodermal Alterations Induced by Dimethyl Sulfoxide.* (31235)

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Intraperitoneal administration of dimethyl sulfoxide (DMSO) to pregnant hamsters induces developmental malformations of their embryos(1,2). The most common malformation encountered is cranioschisis, with exencephaly in later stages of embryonic development. The cephalic malformation is easily reproduced, and occasionally all the embryos of a litter depict the same degree of abnormality(2). This suggests (1) a specific effect of DMSO upon development of the cephalic region of the hamster embryo worthy of further investigation. DMSO-induced cranioschisis represents an excellent opportunity for studying the mechanisms of the embryogenesis of this malformation, to be compared with other previously reported mechanisms of the embryogenesis of vitamin A-induced(3,4) cranioschisis and of the defect in man(5,6,7).

Methods. Fifteen pregnant hamsters were

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