seems more likely that the difficulties encountered are related to the establishment of conditions optimum to feeding. Avoiding the use of a membrane relatively impermeable to attractants and feeding stimulants is apparently an important step toward the accomplishment of optimum feeding.

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Psychopathologic Symptoms Induced by Bis-Beta-Aminopropionitrile

Several chemical substances have recently attracted much interest because of their hallucinogenic and tranquilizing effects. The purpose of our investigation was to establish whether bis-β-aminopropionitrile (Bis BAPN) (*1*) should be considered as a psychopathogenic compound.

Rats of the Sprague-Dawley (200 g) and Long Evans (280 g) strains were injected intraperitoneally with different amounts of bis-β-aminopropionitrile varying from 0.01 to 10 g per kilogram of body weight. Dosages of 4 g/kg and above were lethal within 2 to 7 days, whereas amounts below 1 g/kg caused no obvious symptoms. Levels between 1 and 2 g/kg produced the most striking psychopathologic phenomena. Immediately following injection of 2 g/kg, motor inactiveness, hypersalivation, and increased respiration were induced. For the following 48 hours the animals showed no abnormal behavior.

After approximately 2 days, the animals that had received 2 g/kg showed a marked hyperactivity. They moved their heads from side to side and twitched their necks in a manner reminiscent of patients with von Economo's encephalitis. When placed in an open space, they ran backward in a coordinated manner. If pushed forward, the rat counteracted by pushing backwards, sometimes with such a force as to produce a complete "backward somersault." The slightest touch incited a screaming that was not observed in the controls.

In all, about 80 animals were treated, with identical results. This peculiar behavioral pattern persisted for about 14 days, at which time a decline in backward running was noted. The rats moved alternately forward and backward and in the intervals frequently circled as if chasing their tails. The motor hyperactivity and head twitching persisted. The rats have remained in this condition during a 5-month period of observation; they have been able to eat and also to gain weight.

Albino mice were also injected intraperitoneally with bis-β-aminopropionitrile in a concentration of 1.5 to 2.0 g/kg. After 3 days a motoric hyperactivity became evident. The mice frequently ran in circles as if they were chasing their tails. Occasionally they moved backward and twitched their heads, but this behavior was much less pronounced than in the rats. This phenomenon resembles the genetical "waltzing" anomaly in certain breeds of white mice (*2*) and the symptoms produced by injection of β-β-iminodiproprionitrile (*3*).

Interesting psychopathologic symptoms were observed in birds (*Melopsittacus undulatus*) following a single intraperitoneal injection of 2 g/kg of bis-β-aminopropionitrile. On the third day a general hyperactivity was noted. It was characterized by persistent locomotion, excessive courtship, and compulsive eating. Other abnormalities of the motoric system were periodical circular movement and backward walking.

The behavioral pattern of fish (*Lepomus gibbosus*) can also be changed by intraabdominal injection of 2 g/kg of bis-β-aminopropionitrile. After a delay of 10 days, the fish showed periods of hyperactivity lasting for about 5 minutes, consisting of gyroscopic movements, barrel rolling, swimming on the back or on the side, and standing on the head. Afterward, the fish regained a normal position. These episodes can be produced at any time by merely touching the fish.

An exciter effect was also observed in invertebrates. Grasshoppers (*Melanoplus*) were injected intraabdominally with 1 and 2 g/kg of the same compound. When the lengths of their leaps were measured, it was found that they were significantly increased after administration of the weakest concentration. A protozoan (*Tetrahymena*) was given bis-β-aminopropionitrile in a concentration of 1/10,000 in the culture medium. When the speed with which this organism transverses the microscopic field was measured, it was found to be about twice as fast as that of the controls.

In all the tested animals, bis-β-aminopropionitrile induced a hyperactivity. The changes of the motoric system were most pronounced, but an excitation in more complex behavioral patterns, such as eating and courtship, was also observed. In addition to an acceleration of the normal behavior, the compound also produces apparently new and abnormal patterns. These abnormalities are strikingly similar to the symptoms produced by lysergic acid diethylamide (LSD-25) (*4*). Contrary to the transitory action of lysergic acid and diethylamide, the symptoms induced by bis-β-aminopropionitrile persist. The reason may be that the latter compound produces permanent alteration of the neurons of the spinal cord and brain (*5, 6*).

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Effects of Desoxyribonucleic Acid Breakdown Products on Bacterial Population Changes and Virulence

During studies on the transformation of various strains of *Brucella* spp. by highly polymerized desoxyribonucleic acid (DNA) from genetically different strains, it has been observed (*1*) that the addition of desoxyribonuclease (DNase) to DNA-containing broth cultures causes rapid population changes from M (mucoid) or R (rough) to S (smooth). As a rule, initially non-S (avirulent) cultures of pathogenic bacteria do not undergo population changes to S (virulent) *in vitro*—that is, the gradual establishment of spontaneously arising S mutant cells in initially non-S populations is not favored (*2*). However, in susceptible hosts, or in the presence of DNA and DNase *in vitro*, such population changes (non-S to S) occur with many non-S strains (Table 1).

Studies with *Brucella* have demonstrated that the latter selective effects involve the inhibition of growth and the killing of non-S cells by a breakdown product of DNA. The breakdown product responsible for these effects does not

Table 1. Effects of DNA and DNase on population changes in initially nonsmooth bacterial cultures.

Organism inoculated	Medium	% S after 48 hours (*Pneumococcus*) or after 9 days (*Brucella*) at 37°C
Brucella abortus M	Control (beef extract broth)	< 0.01
B. abortus M	DNA* + DNase	65
Pneumococcus V-497 R	Control (brain-heart infusion broth)	< 1
Pneumococcus V-497 R	DNA + DNase	89
Pneumococcus (99.99% I-192 R + 0.001% ISVI)	Control	0
Pneumococcus (99.99% I-192 R + 0.001% ISVI)	DNA	6
Pneumococcus (99.99% I-192 R + 0.001% ISVI)	DNase	< 1
Pneumococcus (99.99% I-192 R + 0.001% ISVI)	DNA + DNase	100

* DNA from either *Brucella abortus* M or S, *Escherichia coli*, or *Pneumococcus* R.

result directly from the depolymerization of DNA by DNase but requires the additional action of S cells. Thus, the active material, which inhibits non-S cells but does not affect the growth of S *Brucella* cells, is the product of reactions that can be expressed as follows:

DNA + DNase → X + action of S cells → selective factor

These events were verified by several methods, including the demonstration that filtrates from S cultures that contained DNA and DNase inhibited the growth of non-S cells, whereas DNA and DNase alone did not affect the growth of such non-S cells in cultures devoid of S mutants. The source of the DNA apparently has no influence on these selective effects, for DNA isolated from either S or non-S strains of *Brucella*, from *Escherichia coli*, or from pneumococci had the same effectiveness. Inactivation studies on active filtrates indicated that the selective factor might be a nucleotide, yet none of the commercially available nucleotides, nucleosides, purines, pyrimidines, or any of their derivatives that were tested displayed any similar selective effects.

The only chemically known compound that produces similar selective effects is 6-furfurylaminopurine, or kinetin (*3*), a cell-division factor for plants. Kinetin, when it was used in concentrations below 1 μg/ml, also stimulated the selective establishment of S cells in many initially non-S populations; optimum effects were obtained at levels between 0.01 μg and 0.0001 μg/ml. However, a number of M and R strains proved resistant to the effect of kinetin, yet were susceptible to DNA and DNase, and vice versa, thus indicating that the unknown active breakdown product of DNA and kinetin are not identical. Furthermore, upon continued testing, the activity of kinetin became irregular, partially as a result of kinetin contamination of glassware; at the same time, the effects of DNA and DNase remained consistent.

In certain media, supplementation with DNase alone sufficed to promote non-S to S population changes of various *Brucella* cultures. These effects were found to be independent of differences in extracellular DNA accumulation, which occur when *Brucella* cells are grown in different media. Instead, such effects were found to be associated with highly significant differences in the ability of DNase to depolymerize DNA in different, apparently equally complex, media (*4*). Similar environmental effects on DNase activity recently were reported by Catlin (*5*).

The unique selective activities of DNA breakdown products and of kinetin are not restricted to *Brucella* cells; comparable effects have been found in recent studies with pneumococci (*6*). Here too the presence of DNA and DNase (150 μg + 75 μg/ml) favors the rapid establishment of virulent (encapsulated, nonfilamentous) mutants in growing populations consisting initially of avirulent (unencapsulated, nonfilamentous) cells only (Table 1). In the case of some nonsmooth pneumococcal strains (for example, R36A or I-192 R), the initial presence of a few S cells (<0.001 percent) is required to initiate population changes toward S (Table 1); presumably the spontaneous rate of mutation from R to S is exceedingly low in these strains.

The addition of kinetin to pneumococcus cultures has produced effects similar to those described for *Brucella*. Certain R pneumococcus cultures failed to respond to DNA and DNase, but underwent rapid population changes toward S in the presence of a *mixture* of nucleosides, DNA, and DNase. *Single* nucleosides only partially activated the effects of DNA and DNase in these systems. Depending on the strains employed, DNA and DNase either enhance the growth of S cells selectively, inhibit the multiplication of R cells, delay the autolysis of S cells, enhance the autolysis of R cells, or cause a combination of these effects. These selective effects of DNA breakdown products on R to S population changes of pneumococci are independent of the source of DNA employed and should not be confused with the specific R to S transformations obtainable with polymerized pneumococcus DNA (*7*).

Table 2. Effects of DNA and DNase on the virulence of pneumococci (ISVI) for mice.

Animal group*	No. of cells injected per mouse	Percentage dead after					
		24 hr	36 hr	48 hr	60 hr	72 hr	15 days
Untreated	88			0	10	20	20
Untreated	880		0	10	30	40	40
Untreated	8800		0	30	50	60	70
DNA + DNase† at time of infection	88		0	80	100	100	100
DNA + DNase at time of infection	880		0	70	100	100	100
DNA + DNase at time of infection	8800	20	80	100	100	100	100
DNA + DNase 24 hr before infection	88		0	100	100	100	100
DNA + DNase 24 hr before infection	880	0	10	70	100	100	100
DNA + DNase 24 hr before infection	8800	10	70	100	100	100	100
DNA + DNase 24 hr after infection	88				0	100	100
DNA + DNase 24 hr after infection	880			0	10	100	100
DNA + DNase 24 hr after infection	8800			90	100	100	100
Purine pool‡	880			0	10	30	30
Purine pool	8800			40	60	80	80
DNA + DNase, DNA alone, DNase alone, or purine pool				0	0	0	0

* 10 animals per group. † 450 μg of DNA and 200 μg of DNase per mouse. ‡ 3 mg of adenine, 3 mg of guanine, 3 mg of xanthine, and 15 mg of hypoxanthine per mouse.

DNA and DNase not only promoted the establishment of virulent cell types *in vitro* but also exerted an effect on the virulence of S pneumococci *in vivo*. A single subcutaneous (dorsal) administration of 450 μg of DNA and 200 μg of DNase per mouse, either at the time of intraperitoneal infection, 24 hours prior to infection, or 24 hours after infection significantly reduced the survival time and the LD_{50} (Table 2). This virulence-enhancing effect of DNA and DNase appears to be quite different from previously reported effects of purines on the virulence of purine-requiring mutants of *Salmonella, Erwinia, Klebsiella,* and *Agrobacterium* (*8*), for the injection of a purine pool did not affect the survival time of mice infected with pneumococci.

In studies with *Brucella*, desoxyadenosine and sonic extracts of S *Brucella* cells have shown some antagonistic activity toward the S-selecting effects of DNA and DNase *in vitro*. Further studies with these and other antagonists, as well as studies on the chemical nature of the active DNA breakdown product and its mode of action, are now in progress (*9*).

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Paper Electrophoresis of Avian and Mammalian Hemoglobins

As was reported previously (*1*), chick hemoglobin reveals two components on electrophoretic analysis. These components are present in varying proportions, depending on the age of the chick. In view of these findings, it was considered of interest to investigate the electrophoretic behavior of the hemoglobins of birds for comparison with hemoglobins of certain mammalian species. Accordingly, the hemoglobins of the pigeon (*Columba livia*), duck (*Anas*), guinea fowl (*Numida melagris* Linn.), and chick (*Gallus gallus*) and of man (one normal and two cases of Hb-E-thalassemia), cow, goat, and rabbit have been investigated in an LKB paper-electrophoresis apparatus using barbiturate buffer of *p*H 8.6 and of ionic strength 0.05.

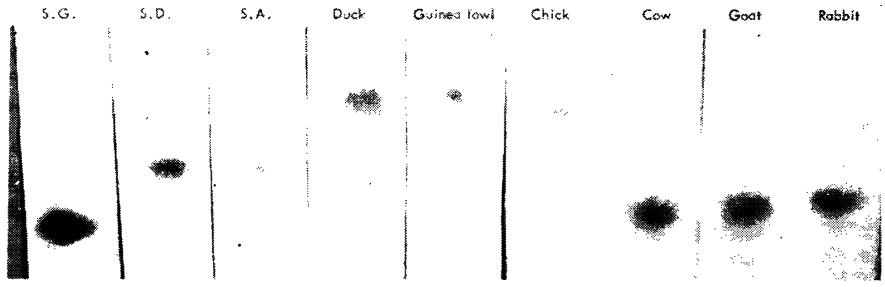

Fig. 1. Paper electrophoresis of avian and mammalian hemoglobins in barbiturate buffer (*p*H 8.6; ionic strength, 0.05) at 220 v applied for 15 hours. Samples S.A. and S.D. were taken from human patients with hemoglobin E-thalassemia; sample S.G. was taken from a normal human adult.

Blood was collected from the jugular vein and was washed with isotonic saline and treated in the usual way (*2*). The hemoglobin solutions thus obtained were centrifuged at 10,000*g* for 15 minutes at 5°C and diluted to a 5-percent solution before electrophoresis. The solutions were kept at −15°C and thawed prior to the electrophoretic runs. The electrophoretic runs were conducted at 220 v for 15 to 18 hours. The electrophoregrams were scanned photometrically at 540 mμ by means of a Photovolt densitometer model 525.

Figure 1 represents the relative positions of the hemoglobins of the rabbit, goat, cow, chick, guinea fowl, duck, two men suffering from hemoglobin E-thalassemia (S.A. and S.D.) and a normal human adult (S.G.).

During this study, it was observed that the blood of the mammals, including the normal human adult, showed only one component (Hb-A), while that of the different birds investigated showed two hemoglobin components. The one moving more slowly toward the anode may be called component 1 and the one moving faster may be called component 2. The percentage composition of each of the two components was evaluated from the density curve by means of a planimeter, and it was found that the proportion of the component 2 was always less. None of the hemoglobin components of the avian blood is identical with the mammalian hemoglobins. Component 2 of avian hemoglobin appears to be identical with hemoglobin E—that is, the special hemoglobin component which is present in the blood of the patients with hemoglobin E-thalassemia. Confirmation that the slower moving component in the blood of these patients referred to here is hemoglobin E (*3*) has been provided independently (*4*). The proportions of components 1 and 2 in avian blood vary from one species to another. The relative mobilities of hemoglobin A in cases of rabbit, goat, cow, and the human beings S.A., S.D., and S.G. were found to be 3.4, 3.6, 3.6, 3.5, 3.5, and 3.6, respectively. The relative mobilities of component 1 and component 2 in chick, guinea fowl, duck, and pigeon blood were found to be 1.0, 1.0, 1.0, and 0.7, and 2.5 2.5, 2.5, and 1.2, respectively. The relative mobility of hemoglobin E in the two cases referred to here was found to be 2.5. The relative mobilities were calculated as centimeters per volt, per second.

Experiments on the rates of alkaline denaturation of the hemoglobins of different species as carried out according to the technique of Singer *et al.* (*5*) indicate that there is no special relationship in this respect between the mammalian and the avian hemoglobins. Even the closely related groups, such as chick and guinea fowl, which are classified under the same order, show varying resistance to alkaline denaturation, the chick hemoglobin being more resistant than guinea-fowl hemoglobin.

Further electrophoretic studies on the hemoglobins of birds such as koel (Cuculidae) and parakeet (Psittacula) revealed the presence of only one hemoglobin component which corresponds to the component 1 of avian hemoglobin, whereas the crow (Corvidae) hemoglobin behaves similarly to the chick hemoglobin. Although the frog and the chameleon hemoglobins undergo a great deal of denaturation during the electrophoretic runs, they reveal the presence of two components. Comparative study of avian hemoglobins by means of paper electrophoresis provides us with the relationship and the evolutionary trends maintained in the chief groups of the birds (*6, 7*).

It is of interest to speculate whether the presence of hemoglobin E in thalassemic patients and in the different avian species is an indication of a common ancestry of mammals and birds, hemo-