toxicity of the *l*-arterenol is reduced when the animals are kept in individual cages, as shown in the last column of the table.

The arterenol toxicity curves are quite flat, as is indicated by the relatively large standard errors. These figures indicate that for equivalent pressor doses *l*-arterenol has a safety ratio (toxicity to pressor activity) which is approximately 4 times that of *l*-epinephrine. This is in agreement with the earlier results (*7*) on the racemic mixture.

Now that *l*-arterenol has been obtained and can be made available for physiological experimentation, it is expected that complete elucidation of its role in the mediation of sympathetic functions and application to therapeutics will follow promptly.

References

1. Bacq, Z. M. *Ann. Physiol.*, 1934, **10**, 467.
2. Bacq, Z. M., and Fredericq, H. *Arch. intern. Physiol.*, 1935, **40**, 454.
3. Barger, G., and Dale, H. H. *J. Physiol.*, 1910, **41**, 19.
4. Crismon, J. M., and Tainter, M. L. *J. Pharmacol.*, 1938, **64**, 190.
5. Greer, C. M., *et al.* *J. Pharmacol.*, 1938, **62**, 189.
6. Stehle, R. L., and Ellsworth, H. C. *J. Pharmacol.*, 1937, **59**, 114.
7. Tainter, M. L. *Arch. intern. Pharm. Therap.*, 1931, **41**, 365.
8. von Euler, U. S. *Acta Physiol. Scand.*, 1946, **12**, 73.

Nitrogen Mustards in Fowl Leucosis[1]

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The recent reviews by Bodansky (*1*) and by Gilman and Philips (*3*) indicate that nitrogen and sulfur mustards elicit a variety of systemic pharmacological actions, chief of which is their ability, in some unexplained manner, to produce death of cells. The generalization is made that cellular susceptibility is related to proliferative activity. The action of the mustards on the blood-forming organs as reflected in the peripheral blood of both experimental animals and man results in a lymphocytopenia, granulocytopenia, thrombocytopenia, and moderate anemia. Because of the marked effects of the mustards on lymphoid tissue, coupled with the finding that actively proliferating cells are selectively vulnerable to the cytotoxic action of the mustards, therapeutic use of these compounds in the treatment of neoplasms of lymphoid tissue in fowls was undertaken. The use of nitrogen mustards in such diseases as Hodgkin's disease, lymphosarcoma, and leucemia in man was sufficiently encouraging to warrant studies with similar diseases of animals (*4, 6*).

Leucosis of fowls is a disease somewhat similar to leucemia of mammals. In fowls the disease is transmissible, and the causative agent is recognized as a filtrable virus. Several forms of the disease occur, depending upon the type of proliferating cells in predominance and upon the tissues involved. The various forms have been designated as lymphomatosis, which may be neural, visceral, ocular, or osteopetrotic and may be leucemic, subleucemic, or aleucemic; and leucosis, which may be erythroblastic or granuloblastic (*9*).

The birds used in these experiments were purchased as day-old chicks from a nearby hatchery and were infected artificially when they were from 1 to 2 weeks of age.

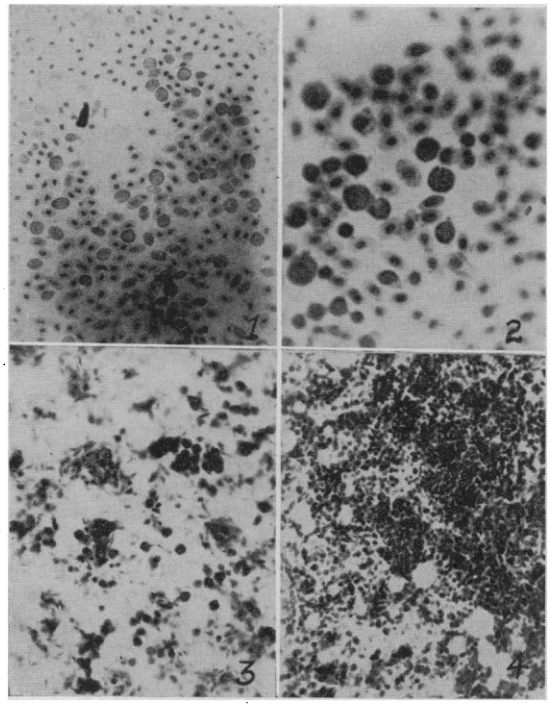

Fig. 1. (1) Photomicrograph of blood smear from chicken with hemocytoblastic leucosis, stained with Wright's stain (×368). (2) Same as (1) at higher magnification (×750). A marked reduction in numbers of these large immature cells was noticeable as early as 24 hrs following treatment with the nitrogen mustards. Note one cell in mitosis. (3) Photomicrograph of femoral marrow from a chicken 4 days after receiving a toxic dose of mustard (×368). Note depletion of lymphoid cells. (4) Photomicrograph of femoral marrow from a normal chicken of the same group and of the same age. Note hyperplasia in contrast to (3).

About equal numbers of the Barred Plymouth Rock and New Hampshire Red breeds were used. The Beltsville strain "A" leucosis virus was used throughout (*5*). When week-old chicks were injected intravenously or intraperitoneally with blood containing this strain of virus, they usually developed an acute form of erythrogranuloblastic leucosis in from 4 to 6 weeks. The injected birds that did not develop an acute form usually later developed either a chronic visceral form characterized by great enlargement of the liver, spleen, and other visceral organs or the nerve form characterized by cellular infiltration of nerves, and paralysis (*10*). The

[1] The nitrogen mustards were obtained through the courtesy of the Medical Division of the Army Chemical Center.

acutely affected birds usually developed an overwhelming preponderance of immature cells in the peripheral blood, referred to by Jordan (*8*) as hemocytoblasts, and were in effect cases of hemocytoblastic leucosis (Fig. 1, 1 and 2). Of these that were treated with the mustard compounds there were 15 that had acute hemocytoblastic leucosis and 13 that had neural lymphomatosis. Four had visceral lymphomatosis characterized by enormous enlargement of the liver as determined by rectal palpation. In this form any of the visceral organs may be diffusely infiltrated with lymphoid cells, or there may be multiple tumor masses made up chiefly of these cells (*7*).

One was a case of ocular lymphomatosis in which the iris of the affected eye was densely infiltrated with lymphocyte-like cells (*10*).

The preliminary work consisted of a determination of the approximate optimal and safe dose of the two mustard derivatives used, namely, methyl-*bis*(β-chloroethyl)-amine (HN_2), and *tris*(β-chloroethyl)amine (HN_3). This was accomplished by injecting normal chickens weighing approximately 0.5 kg each with varying amounts of the mustards. Ten such birds were injected intravenously (*vena ulnaris*) with a gradually increased dose of the HN_2 compound per bird, ranging from 0.5 to 10 mg/kg body weight, each dose being dissolved in 2 ml of sterile physiological sodium chloride solution immediately before administration. In a similar manner, 10 comparable birds were injected with the HN_3 compound. It was found that, when from 3 to 5 mg/kg of HN_2 was given, there was drooling of fluids from the mouth, some diarrhea that persisted for about 24 hrs, and evidence of leucopenia without lethal results. The HN_3 compound was found to be about twice as toxic as the HN_2. In general, the symptoms produced by the two compounds were similar. There seemed to be considerable individual variation in the reaction of birds to both compounds. From these experiments with each compound on 10 normal birds, weighing about 0.5 kg each, it appears that the optimal dose of HN_2 is approximately 2 mg/kg, and of HN_3, approximately 1 mg/kg. At autopsy of the birds that died 24–72 hrs after treatment with either of these compounds, the spleen was found to be considerably shrunken and the femoral marrow was of a yellowish color, denoting depletion of hemopoietic cells. Suitable portions of heart, liver, spleen, pancreas, duodenum, sciatic nerve, femoral marrow, and thymus from these birds were fixed in Helly's fluid, sectioned at 5 μ, and stained with Delafield's hematoxylin and azur-eosin. Microscopic examination of these revealed marked atrophy of lymphoid tissue in spleen, thymus, duodenum, and femoral marrow. The most pronounced microscopic lesions after a lethal dose were observed in the marrow, spleen, and intestines. In the marrow there was a marked decrease in numbers and in mitotic activity of hemopoietic cells. This depletion of the marrow progressed until the sinusoids contained only a few mature cells, and if the bird lived for a few days after the treatment, there was almost complete aplasia (Fig. 1, 3 and 4). A similar depletion of lymphoid cells occurred in the spleen. The intestinal lesions consisted chiefly of vacuolization of epithelial cells and atrophy of adjacent lymphoid tissue.

The birds with hemocytoblastic leucosis contained enormous numbers of immature cells (hemocytoblasts) in their peripheral blood when treatment was started. In the cases that responded to treatment there was a marked reduction in these cells 24 hrs after treatment. In two such leucotic birds that were killed one week after receiving the mustards, the spleen and liver were practically of normal size. These organs are invariably enlarged in this form of leucosis. Likewise, in two birds with marked enlargement of the liver as determined by rectal palpation, there was practically complete reduction to normal size of this organ two weeks following one treatment with the HN_2 compound. Six birds with neurolymphomatosis responded to treatment and made a complete clinical recovery. Most of the birds that recovered, especially when more than one treatment was necessary, developed a transient leucopenia and slight anemia that was noticeable about two weeks after treatment.

Fourteen birds infected artificially with strain "A" leucosis were treated with HN_3 and 19 similarly infected birds were treated with HN_2. Two of these, in the early stages of the hemocytoblastic form when treated with HN_3, made clinical recoveries lasting from 3 to 6 months following the last treatment. Three with hemocytoblastic leucosis, 6 with neurolymphomatosis, and 2 with visceral lymphomatosis when treated with HN_2 have made complete clinical recoveries. In addition, 7 spontaneous cases were treated. One of these was a case of the visceral form treated with HN_3 without response. Three with neural and 3 with the visceral form were treated with HN_2. One of each showed improvement but recurred. One of the visceral cases made a complete recovery lasting 8 months. The others did not respond.

The results, while not highly impressive, appear to indicate that, if these compounds are given in the early stages of the disease, the results are better and more apt to be of a permanent nature. In advanced cases the effects were only temporary, and a recurrence of the disease usually resulted in death of the bird. There may have been several factors that played a part in the results obtained. Smaller doses repeated more frequently, as well as the use of larger quantities of saline to dilute these mustards prior to injection, should be tried. The effect, if any, upon the blood vessels should be studied to learn if any permanent injury occurs. It is interesting to note, however, that in 9 birds out of 33, clinical recovery resulted after one treatment.

In some instances, especially when repeated treatments seemed necessary, intravenous injections were difficult to accomplish. This was due to the irritation set up from some of the mustard escaping into the subcutaneous tissues. Because of the marked irritation and subsequent formation of scar tissue around the vein, it is important to exercise care in making the injections to avoid damage to the sites for future intravenous therapy.

The work related herein indicates that the two nitrogen mustards used have a profound action upon the immature

cells spoken of as hemocytoblasts in a highly fatal disease of fowls. In many instances a retardation of mitotic activity both in the marrow and in the peripheral blood was demonstrable 24 hrs after treatment. Some evidence to indicate that these mustards have a lethal effect upon the causative virus in addition to causing death of the proliferating cells was indicated by the failure of blood drawn from treated birds to transmit the disease.

References

1. BODANSKY, OSCAR. *Science*, 1945, **102**, 517–521.
2. FURTH, J. *J. exp. Med.*, 1931, **53**, 243–267.
3. GILMAN, ALFRED, and PHILIPS, F. S. *Science*, 1946, **103**, 409–415, 436.
4. GOODMAN, L. S., WINTROBE, M. M., DAMESHEK, W., GILMAN, A., and MCLENNAN, MARGARET T. *J. A. M. A.*, 1946, **132**, 126–132.
5. HALL, W. J., BEAN, C. W., and POLLARD, MORRIS. *Amer. J. vet. Res.*, 1941, **2**, 272–279.
6. JACOBSON, LEON O., SPURR, CHARLES L., BARRON, E. S., GUZMAN, SMITH T., LUSHBAUGH, C., and DICK, G. F. *J. A. M. A.*, 1946, **132**, 263–271.
7. JOHNSON, E. P. *Va. agric. exp. Sta. Tech. Bull. 44*, 1932.
8. JORDAN, H. E. *Amer. J. Anat.*, 1919, **25**, 437–479.
9. JUNGHERR, E. *Amer. J. vet. Res.*, 1941, **2**, 116.
10. PAPPENHEIMER, A. M., DUNN, C. C., and CONE, V. *Storrs agric. exp. Sta. Bull. 143*, 1926.

In Vitro Studies of Caries of the Enamel in the Syrian Hamster

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In previous pathological studies of enamel caries in the human (*1*, *2*) and hamster (*4*) it was observed that two morphological types of microorganisms were constantly associated with the microscopic plaque and the early enamel lesion. Thread forms predominate in the more superficial areas of the plaque, whereas spheroidal forms were always present in the affected enamel beneath the plaque. As a follow-up of this observation, it seemed advisable to attempt to isolate and culture these organisms.

Scrapings were taken from the plaque overlying the early carious area, in which the first evidence of enamel caries is the appearance of a brown-stained spot in an apparently intact enamel surface. The scrapings were cultured, and a growth of filamentous forms was obtained which resembled those found predominating in the plaque of the earlier histological sections. These organisms developed on blood agar after incubation at 37° C for 4–5 days. An atmosphere of about 10% carbon dioxide seemed to be favorable for primary isolation. Subsequently the organisms will grow on a variety of media. Fermentation of the carbohydrates tested in the growth of the organisms was slow with the different strains tested. Gelatin was not liquefied, but the colonies developed a dark brown pigment in 1–2 weeks in this medium.

These organisms are similar to the actinomycetes, a group which has been called simple molds or pleomorphic bacteria. They are easily identified with some of the *Leptothrix* and *Cladothrix* described in the earlier literature under terms no longer used. Except for their more aerobic habits and the ease with which they are cultivated, they correspond closely with some strains isolated by several workers from normal mouths and throats, from carious detritus, gingivitis, and cervicofacial actinomycosis, and classified as *Actinomyces bovis* or *A. israeli*.

A high degree of pleomorphism is characteristic of these isolated filamentous organisms. Various small forms resembling diphtheroids, short bacilli, and irregular cocci, as well, were frequently observed to be associated with the filamentous cells in a single colony. After repeated subculture, some of these associated forms almost completely replaced the filaments. The change in the appearance of the filamentous types to other morphological forms may be accompanied by a change in the appearance of the colony, but this is not always the case. The filamentous colony is rough, whereas when transition to other forms takes place, the colony may have a smooth, glazed appearance. Neither the age of the culture nor the type of media has thus far been shown to be associated with the change in the morphological appearance of the cells. While the various cell types of the colonies most likely represent a single highly pleomorphic species, the possibility of a symbiotic relationship between two or more species which are difficult to separate in culture could not, at this point, be entirely ruled out.

Isolated filamentous cells growing on laked blood agar were selected for study and their multiplication was recorded with photomicrographs at definite intervals of time (Fig. 1). These observations were made at 2-hr

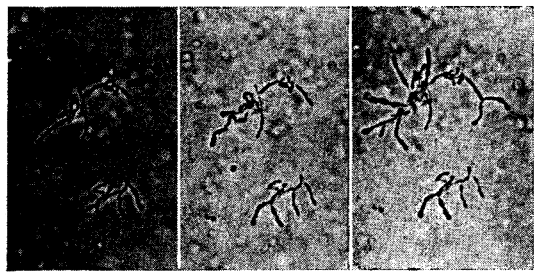

FIG. 1. Illustration of the filamentous organisms selected for microscopic study at incubation of: A, 7 hrs; B, 9 hrs; C, 13 hrs.

intervals for periods up to 33 hrs. In order to prevent this preliminary study from becoming too prolix, the experiment was limited to a relatively stable strain of the isolated organisms.

In the cultures of a single organism, the filaments were observed to divide into short bacillary bodies. Some of these in time elongated into filaments. Terminal or lateral branches were frequently found on the lengthening filaments. Occasionally a whole segment of a filament appeared to undergo autolysis, while the adjacent portion continued to proliferate. As the colony continued