Table 1. Distribution of Hæmoglobin Types in Five Populations of Wild Mice

Population	Number of mice	Hb^s type	Hb^d type
T I	6	2	4
T II	7	6	1
T III	15	1	14
H I	6	0	6
H II	7	6	1
Additional	1	1	0
Total	42	16	26

Gerald's[2] suggestion that Hb^d may be more widespread in Nature. However, individual populations differed widely from one to another.

Furthermore, studies of mutants[1,5] show that animals with the Hb^s type can arise as mutants from Hb^d lines and possibly vice versa. Mutant strains can also be found with some animals of the single and some of the diffuse type[1,9]. We therefore do not always know for certain that the wild ancestors of laboratory strains already possessed the Hb^s allele. In the strain '*CE*', which originated from a wild mutant (Knight[10]), we found the Hb^s type in animals of the line *CE/J* Han Jena (F_{25}).

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Effects of Erythromycin and Tetracycline in Human Bone-marrow Cultures

In a previous publication[1] we have reported that chloramphenicol inhibits hæmoglobin synthesis in human bone-marrow culture. Erslev and Iossifides[2] found that not only chloramphenicol but also tetracycline and erythromycin had similar effects on immature erythrocytes *in vitro*. Because tetracycline and erythromycin do not cause toxic hæmatological reactions in man, this finding would seriously limit the validity of applying this *in vitro* method to the study of the effect of chloramphenicol on erythroid cells *in vivo*. It seemed important, therefore, to extend our experiments and to study the effect of tetracycline and erythromycin on hæmoglobin synthesis in human bone-marrow cultures, using the same methods as were used to study the effect of chloramphenicol.

The detailed method has been described previously[1]. Bone marrow was obtained by aspiration from the sternum or iliac crest. Cell suspension cultures were prepared under aseptic conditions, using Hanks's solution and 40 per cent autologous serum as medium. ^{59}Fe in the form of $^{59}FeCl_3$ (Abbott Laboratories, Ltd., Montreal) (specific activity, 4·4–11 μc./μg) was added to the cultures to a final concentration of 0·01 μg/ml. The amount of iron added never exceeded the iron-binding capacity of the serum. Crystalline chloramphenicol (Parke Davis, Ltd., Montreal), erythromycin (Abbott Laboratories, Montreal) and tetracycline (Pfizer Canada Chemicals, Montreal) were dissolved in Hanks's solution and added, in various amounts, to the cell suspensions. The maximum concentration of the three antibiotics in the culture was 500 μg/ml., 500 μg/ml. and 100 μg/ml., respectively. The low solubility of the pure crystalline tetracycline did not allow the use of higher concentration. The volume of the cultures was 2 ml. in each tube and the total nucleated cell count varied from 2–4 × 10^6 per ml. The cultures were rotated at 12 r.p.h. for 24 h at 37° C. At the end of the incubation period, they were centrifuged, the cells washed three times with physiological saline and hæmolysed with distilled water at 4° C.

The radioactivity of the total hæmolysate was then measured in a well-type scintillation counter. From this, the total Fe uptake was calculated and expressed in μg Fe/erythroblast/h.

After centrifuging the tubes containing the hæmolysates at 3,000*g* for 15 min, the supernatant was removed and its radioactivity determined. From this, the amount of Fe incorporated into hæm was calculated and also expressed in μg Fe/erythroblast/h.

Cultures containing no added antibiotics, but prepared from the same marrow specimen, served as controls. In each experiment, cultures were prepared in duplicate.

As noted in our previous communication, when chloramphenicol was added to the cultures, iron uptake by the erythroblasts was slightly decreased, whereas iron incorporation into hæm was markedly decreased. Therefore, iron utilization, that is, the fraction of iron utilized for hæmoglobin synthesis in relation to the amount taken up by the cells, was significantly lower in the presence of chloramphenicol. This effect was proportional to the concentration of chloramphenicol in the culture, and the lowest concentration which gave consistent results was 500 μg/ml.

The addition of erythromycin to the cultures had a different effect. This was consistent in cultures of five different bone marrows, and Table 1 shows one representative experiment. The amount of iron taken up by the erythroblasts was strikingly reduced in the presence of erythromycin. Consequently, less iron was found in the hæmolysate. However, in contrast to chloramphenicol, in the erythromycin-treated cultures the percentage of the iron taken up by the cells which was incorporated into hæm was the same as in the controls. Thus, iron utilization was not altered in the presence of erythromycin. The effect of erythromycin depended on its concentration in the culture and 250 μg/ml. was found to be the smallest effective dose. 500 μg/ml. further reduced iron uptake, but had no influence on iron utilization.

Table 1. Effects of Erythromycin and Chloramphenicol on Iron Uptake and Iron Incorporation by Erythroblasts in Bone Marrow Suspension Culture

Incubation time 24 h. Values of a representative experiment	Control	Chloramphenicol 500 μg/ml.	Erythromycin 250 μg/ml.
Fe uptake, × 10^{-9} μg Fe/cell/h	1·59	1·12	0·61
Fe incorporation	0·79	0·21	0·39
Percentage of iron uptake incorporated into hæm	49·7%	18·8%	63·9%

The effect of tetracycline on erythroblasts was studied in 14 bone-marrow cultures. When 50 or 75 μg/ml. was present in the medium, the iron uptake and incorporation by the erythroblasts were not altered as compared with the controls. When 100 μg/ml. tetracycline was used, the uptake of iron by the erythroblasts was decreased in four of seven cultures but, as with erythromycin, the percentage of iron which was incorporated into hæm was the same as in the controls. Hence, both erythromycin and tetracycline decreased iron uptake but had no effect on iron utilization by erythroblasts in these bone-marrow cultures. No relationship could be found between the susceptibility of the cultures to the toxic effect of these agents and the hæmatological status of the patients from whom the bone marrows were obtained.

These observations indicate that chloramphenicol, erythromycin and tetracycline do not have identical effects on erythroblasts cultured *in vitro*. Chloramphenicol

slightly diminished iron uptake by the erythroblasts and interfered markedly with iron incorporation into hæm. On the other hand, both erythromycin and tetracycline depressed iron uptake by the cells. The mechanism of this effect is not clear, but, as tetracycline is known to be a chelating agent[3], a possible explanation is that it binds iron, thus making it less available for the cells. Of the iron taken up by the erythroblasts, the same percentage was incorporated into hæm as in the control cultures. Since this step is related to hæm synthesis, it seems that, in contrast to chloramphenicol, neither erythromycin nor tetracycline interferes with this synthesis in the erythroblasts. Erslev and Iossifides[2] did not find this different pattern of behaviour *in vitro* and, therefore, concluded that there was a discrepancy between the *in vivo* and *in vitro* observations. In contrast, the difference in the action of these drugs *in vitro* in the experiments reported here may explain their different behaviour *in vivo* in relation to the hæmic cells. Also, in our experiments, tetracycline affected erythroblasts to a lesser degree than did erythromycin or chloramphenicol, which may be related to its lower concentration in the cultures.

The effective *in vitro* concentration of the three drugs in relation to their *in vivo* therapeutic blood level was also different. The 500 μg/ml. concentration of chloramphenicol is 10–15 times[4], the 100 μg/ml. tetracycline is 20–40 times[5,6] and the 250 μg/ml. erythromycin is 50–100 times[7,8] the average effective therapeutic blood level. Lower concentrations of erythromycin and tetracycline, corresponding to 10–15 times their effective therapeutic blood level, had no measurable effect in the cultures. The fact that relatively higher concentrations of tetracycline and erythromycin were necessary to cause an adverse effect *in vitro* may also reflect that the hæmic cells are less sensitive to these drugs.

Finally, the fact that the effects of the three antibiotics on iron metabolism *in vitro* were different suggests that their antimicrobial action may also be different. This is in agreement with Brook[9], who suggested that, although their action on bacteria is assumed to be similar, based on the inhibition of protein synthesis, this cannot be more than a superficial similarity, in view of their completely different chemical structure. It must be recognized, however, that the action of a drug on a bacterium, and its action on the human cells, may be quite unrelated.

In summary: (*a*) Both erythromycin and tetracycline decreased the iron uptake by the erythroblasts, but had no effect on the utilization of iron for hæm synthesis. This indicates that, in contrast to chloramphenicol, these antibiotics did not interfere with hæm synthesis under the same experimental conditions. (*b*) The difference between the effects of these antibiotics *in vitro* may be related to their different behaviour *in vivo* on the hæmic cells.

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IMMUNOLOGY

Suppression of Experimental Allergic Encephalomyelitis in Rats and Chickens treated with Reserpine

Previous investigations have shown marked suppression of delayed hypersensitivity reactions, prolongation of skin homograft survival, and impairment of antibody production in rats treated with reserpine[1]. Moreover, this *Rauwolfia* alkaloid was regularly found to produce thymic involution. This communication is an extension of these investigations, and deals with the effect of reserpine on the development of experimental allergic encephalomyelitis in the rat and chicken.

Adult male albino rats weighing 220–260 g from a randomly bred colony were treated with intraperitoneal 0·5 mg/kg injections of reserpine ('Serpasil', Ciba, Basle) for 17 consecutive days. Three days after the beginning of treatment all rats were injected with an emulsion containing fresh normal rat spinal cord and complete Freund's adjuvant[2]. Each rat received a single inoculation of 0·1 ml. of this antigen–adjuvant mixture in the left hind foot-pad. Control animals were treated with 17 injections of saline, and immunized with spinal cord adjuvant mixture as described for reserpine-treated rats. Animals were observed daily for the onset of clinical signs of disease. Those rats which showed paralysis or ataxia were killed on the day of onset of symptoms; the remaining rats were killed 19 days after immunization. Paraffin sections of cerebrum, cerebellum, medulla and three levels of spinal cord were prepared, and stained with hæmatoxylin and eosin. Histological changes were graded from 0 to +++[2]. Rats from both experimental and control groups were weighed every three days. At autopsy the thymus was also weighed.

Rhode Island Red chickens were treated with 2 mg/kg reserpine intraperitoneally every three days, beginning one day after hatching. At eight weeks, each chicken received foot-pad injections of homologous spinal cord adjuvant mixture as described elsewhere[3]. Control chickens of exactly the same age were immunized in an identical manner. Reserpine-treated and control birds were skin-tested 14 days following immunization; for this purpose 0·1 ml. containing 50 μg of chicken spinal cord lipid, obtained by petroleum-ether extraction, was injected into the left wattle. The right wattle was injected with 0·1 ml. of a 1 : 5 dilution of old tuberculin. The reactions were read at 24 h, and graded from 0 to +++ on the basis of œdema, induration and discoloration of the wattle. Sections of cerebrum, cerebellum, medullary bulb and spinal cord at three levels were stained with hæmatoxylin and eosin, and lesions were classified from 0 to +++[3]. Thymic lobes, spleen and bursa of Fabricius of all chickens were weighed at the end of the experiment.

The ability of reserpine-treated rats to develop experimental allergic encephalomyelitis was strikingly suppressed (Table 1). Eight of 10 rats injected with 0·5 mg/kg of reserpine showed no histological changes in the central nervous system, whereas 8 of 12 controls exhibited definite lesions characteristic of disease, and two rats of the latter group developed paralysis.

Table 1. Suppression of Experimental Allergic Encephalomyelitis in Rats treated with Reserpine

Group	No. of rats	No. of rats with encephalomyelitis* 0	+	++	+++	Average weight Thymus (mg)	Body (g)
Reserpine-treated (0·5 mg/kg)	10	8	1	1	0	90·0	129·7
Control	12	4	3	3	2	217·3	160·0

* Average severity of lesions observed in spinal cord, medulla, cerebellum and cerebrum.