FURTHER EXPERIMENTS ON THE GOLDEN HAMSTER (*CRICETUS AURATUS*) WITH TUBERCLE BACILLI AND THE VOLE STRAIN OF ACID-FAST BACILLUS (WELLS)

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The experiments on the golden hamster with the three types of tubercle bacilli and the vole strain of acid-fast bacillus previously reported (Griffith, 1939) showed that this species of animal was susceptible to infection with all four types of acid-fast bacilli. Three of the types (bovine, human and vole) each produced macroscopic generalized tuberculosis which differed in degree of severity. Of these types the bovine was the most virulent, producing extensive necrosis or caseation of the lesions, and the vole strain the least, the lesions showing no caseation or necrosis. The result with the avian bacillus (only one hamster was available) differed in kind from those of the others; the bacilli multiplied enormously in the local gland and some of the organs, but did not form macroscopic tuberculous lesions; the histological picture was comparable with avian tuberculosis in other animals.

Through the kindness of Mr R. E. Glover, F.R.C.V.S., Director of the Farm Laboratories of the National Institute for Medical Research, who supplied me with an additional twelve golden hamsters from the stock bred at the Farm, I was able to make further experiments on this species, mainly in test of the virulence of the types by feeding and of the effects of smaller doses of avian bacilli.

The details and results of the experiments are summarized in Table 1. The hamsters were fed by placing the mass of culture on the tongue by means of a platinum spatula.

Distribution and characters of the lesions

Subcutaneous Inoculations

Hamster 9 (human strain). The local lesion measured $2 \times 2 \times 0{\cdot}95$ cm. and was in the abdominal muscles; it was composed of somewhat firm greyish semitranslucent homogeneous substance showing a streak or two of necrosis or caseation under $\times 8$ (t.b.++++). The left inguinal, left axillary, both iliacs, both renal (t.b.++++) and both ventral mediastinal glands (t.b.++++) were considerably enlarged, of a softish consistency, hyperplasic and homogeneous, except some which showed opaque yellowish rather diffuse patches in the cortices. Other glands were slightly enlarged but not opaque anywhere. Spleen enlarged, showed enlarged Malphigian bodies and

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granulation tissue in the hilum (t.b. + +). Liver enlarged, closely beset with grey translucent miliary tubercles, some aggregated and most of them showing one or more microscopic white speckles (t.b. + +). Lungs, some red areas of hepatisation and a moderate number of translucent grey tubercles, up to 1·5 mm., few only showing small opacity (t.b. + +). Kidneys normal.

A culture of human type was obtained from a submaxillary gland.

Table 1

No. of hamster	Strain of bacilli	Dose of culture mg.	Duration of life days	Results
			Subcutaneous inoculations	
9	Human (sputum)	1·0	K. 71	General tuberculosis
15	Nigerian strain 85	1·0	D. 74	
10	Avian	1·0	D. 253	Local lesion only. T.b. disseminated, not numerous
11	Avian	1·0	D. 455	No macroscopic tuberculous lesion. T.b. in liver + +
17	Avian	5·0	D. 154	No local lesion. Liver and kidneys, moderate number greyish foci
20	Vole	1·0	D. 305	Generalized lesions in which bacilli abundant; no caseation
			Feeding	
16	Bovine 33	10·0	D. 68	General tuberculosis, not severe
13	Human (sputum)	10·0	D. 62	Slight generalized tuberculosis
18	Human	10·0	K. 518	No macroscopic tuberculosis
14	Avian	10·0	D. 125	No macroscopic tuberculosis. T.b. numerous in mesenteric glands, sparse elsewhere
12	Vole (egg culture)	10·0	D. 104	Submaxillary gland only affected (A.-F.B. + + + +). Acid-fast bacilli disseminated but scanty
19	Vole (potato culture)	10·0	D. 294	No sign of disease

Hamster 15 (Nigerian strain). Local ulcer, 2 × 2 cm. Inguinal glands, left wholly, right partly caseous. One prescapular and one iliac gland each contained a caseous focus. Portal gland wholly, bronchial glands partly caseous. Lungs, moderate number large caseous masses. Spleen, little if at all enlarged, scattered creamy tubercles. Liver, moderately numerous grey foci. Kidneys normal. Suprarenal bodies enlarged and caseous.

Smears: Inguinal gland and spleen, t.b. + + + +, beading marked; liver, t.b. + + +.

Cultures from inguinal gland and liver resembled original.

Histology (by W. Pagel, M.D.). Liver, congested, showed beginning cirrhosis and many large epithelioid cell nodules in peripheral parts of the acini; cells of nodules full of tubercle bacilli and some extracellular; in addition many acid-fast droplets in my (W. P.'s) opinion the products of intracellular digestion of acid-fast material. Spleen, small epithelioid cell nodules in Malphigian corpuscles and in addition some larger foci with caseation; large numbers of tubercle bacilli in nodules and diffusely distributed over the red pulp, chiefly in the surroundings

of the Malphigian corpuscles and mostly extracellular. Lymphatic gland, diffusely caseated and overgrown by bacilli; in peripheral parts epithelioid cell nodules with small lime salt deposits in caseous parts. Lungs and suprarenal body diffusely caseated, caseous parts showing enormous numbers of bacilli, occasional small calcium deposits in lungs.

Hamster 10 (avian strain). At site of inoculation there was a thin tract, 1 cm. in greatest width, of light brownish translucent granulative tissue in which were abundant tubercle bacilli. There was no sign of disease elsewhere. Smear preparations. Left inguinal, right axillary, left submaxillary and mesenteric glands, t.b.+. Spleen, two t.b. seen. Liver and rectal faeces, no t.b.

Cultures: Blood (one drop), one avian colony on each of two tubes; none on a third. Liver, ten and sixteen avian colonies on two tubes. Spleen, moderately numerous avian colonies.

Histology. Owing to bad fixation resulting in autolysis of the tissues, structural changes, etc. were ill-defined. The spleen was apparently full of epithelioid cell nodules in Malphigian corpuscles and the liver showed casual accumulation of large epithelioid cells in the periportal sheath, surrounded by leucocytes. Z.-N. preparations negative, due to autolysis.

Hamster 11 (avian strain). Body fat and in good condition. Scar at seat of inoculation. Lungs, pneumonic condition. Liver, nutmeg appearance. No tubercles elsewhere.

Cultures: Right inguinal gland, numerous avian colonies. Liver, moderately numerous avian colonies.

Histology. Liver, in the periportal sheath hyaline deposits containing, in the centres, mononuclear cells and leucocytes; some of these deposits appeared in nodular form and were probably tubercles although Z.-N. was negative (autolysis). Spleen, nodules consisting of large mononuclear cells, full of acid-fast elements (rods and granules): sinus wall amyloid, red pulp atrophic, no Malphigian corpuscles visible (probably substituted by the nodules described). Lungs congested. Kidney and suprarenal body, no specific changes.

Hamster 17 (avian strain). No trace of local lesion seen. Inguinal glands slightly enlarged, no caseation. Lungs, right pneumonic. Liver and kidneys, moderate number of greyish white or grey foci. No sign of disease elsewhere.

Smear preparations: Right inguinal gland, t.b.+++, mainly short, liver t.b.+, many deep stained forms.

Cultures: Typical avian colonies from inguinal gland (numerous) and from liver (moderately numerous).

Histology. Liver, spleen, kidney and lung extremely congested; no specific changes. Lymphatic gland (? left inguinal) showed large areas consisting of epithelioid cells. Z.-N. preparations negative except that of gland which showed occasional acid-fast bacilli intracellularly. The almost complete failure to stain the bacilli in the tissues was probably due to autolysis following bad fixation.

Hamster 20 (vole strain). Local lesion, on left side tuberculous ulcer covered with dry scab, with yellow necrotic material under partly everted and partly inverted margins. Left inguinal gland, 1·2 × 1 cm. soft and yellowish in parts, but mostly retained gland structure. Right inguinal gland, 0·6 × 0·4 cm., hyperplasic and soft. Left axillary, enlarged, 1 × 0·5 cm., contained several yellow areas (section). Right axillary gland, slightly enlarged, 0·5 cm., soft hyperplasic. Left iliac gland, much enlarged, 0·75 cm., soft, yellowish. Right iliac gland not affected. Portal, thoracic and mesenteric glands enlarged and hyperplasic. Lungs, pink and crepitant, multiple greyish granulations from a pin-point up to 1·5 mm. over both lungs, some aggregated and beginning to coalesce. Liver, closely beset with tiny greyish yellow areas up to 0·5 mm. Spleen, 4 cm. long; numerous grey nodules in substance and projecting from borders; on section mottled grey areas in surrounding reddish bands.

Smears: Local lesion, right inguinal gland, liver, spleen—very numerous acid-fast bacilli, large clumps resembling pure culture.

Feeding

Hamster 16 (bovine strain). Caecum, pinhead tubercle on serous surface. Omentum, one large and a few small tubercles. Mesenteric glands enlarged, showed early caseation. Spleen, five small creamy foci. Liver, scattered to moderate number minute grey foci. Lungs, scattered large creamy tubercles.

Smear preparations: Mesenteric glands, t.b. + + + +, Liver, t.b. + +; thick, deeply stained forms.

Cultures of bovine bacilli were obtained from the mesenteric glands (very numerous colonies) and the liver (moderately numerous colonies).

Hamster 13 (human strain). Structures in neck normal. Caecum, a few whitish specks seen through wall, but nothing from mucous surface. Mesenteric glands, slightly enlarged, hyperplasic, showed on section under magnification a fine network of necrosis or caseation. Portal gland, similar appearance. Tracheobronchial glands slightly enlarged and opaque. Lungs crepitant, showed seven greyish yellow areas, 2 mm. in diameter and about half-a-dozen smaller grey translucent foci down to just visible. Spleen, liver and kidneys, nothing definite.

Smear preparations: Mesenteric, portal and tracheal glands and lung lesion, t.b. + + + +. Caecal mucous membrane, t.b. + +. Spleen, liver and faeces t.b. +.

Cultures of human type recovered from spleen (numerous) and blood. Whole blood was treated with KOH and two colonies were obtained on each of two tubes from one drop.

Hamster 18 (human strain). No sign of tuberculosis.

Cultures: Mesenteric glands negative.

Hamster 14 (avian strain). Peyer's patches in small intestines and lymphoid patches in caecum showed blackish pigmentation but were not obviously enlarged (t.b. + +). Mesenteric glands slightly enlarged, showed blackish

pigmentation in parts (t.b. + + +). Liver, light brownish in colour and lobulation distinct. Other organs were normal (spleen, 2 t.b. only); also lymphatic glands (portal gland, t.b. +; submaxillary glands, no t.b.).

Hamster 12 (vole strain). Left submaxillary gland enlarged, nearly 1 cm. in diameter, hyperplasic and congested, showed (8 ×) minute irregular necrotic foci, not numerous and not regularly distributed. Mesenteric glands not enlarged. No sign of disease elsewhere.

Smear preparations: Submaxillary gland, very numerous typical vole bacilli. Mesenteric gland, acid-fast bacilli + + in groups and single forms. Spleen and liver, no acid-fast bacilli seen.

Hamster 19 (vole strain). No sign of disease.

Smear preparations: Heart blood, spleen and mesenteric gland negative.

Cultures: Heart blood and spleen negative.

Summary

Subcutaneous inoculations: Two strains of human origin were tested, one was of eugonic human type from the sputum of an English case of pulmonary tuberculosis; the other was of dysgonic human type obtained by Dr J. A. Young from a native in Nigeria. Both strains produced typical general tuberculosis in the hamster, the lesions on the whole showing less caseation than those set up by bovine bacilli (vide 1939).

The vole strain of bacillus gave rise to generalized disease resembling tuberculosis. The duration of life of the animal was, however, prolonged and though the bacilli were abundant in the lesions there was no necrosis or caseation. Similar results in two hamsters were reported in 1939.

The avian bacillus was the least pathogenic of the four types. Of the three hamsters inoculated only one (H. 17, dose 5·0 mg.) showed macroscopic foci in the liver and spleen; as these were not seen microscopically in sections it is doubtful if they were due to the action of tubercle bacilli. It is unfortunate that the specimens of Hamster 17, which died in my absence from the laboratory, were badly fixed; a smear preparation of the spleen was not made. In the others tubercle bacilli were found in the organs, but were not numerous. The peculiar result after 5·0 mg. of avian bacilli—enormous multiplication of bacilli in spleen, liver and a local gland without macroscopic tuberculous lesions—described in the first paper was not reproduced.

Feeding: The four different types of bacilli were given by the mouth to six hamsters, the dose in each instance being 10 mg. of culture. Two of the hamsters, one fed with a potato culture of the vole strain, the other with a human strain, escaped infection. The remaining four hamsters, each fed with one of the four types of bacilli, developed disease, the severity of which in each instance was in harmony with that following subcutaneous inoculation of the type.

CONCLUSION

The golden hamster is susceptible, both by subcutaneous inoculation and by feeding, to infection with the bovine, human, avian and vole types of bacilli.

The bovine type of bacillus is the most virulent for the hamster, followed closely by the human type, both of which produce generalized progressive tuberculosis.

The vole type causes generalized tuberculosis of slow development, the lesions consisting histologically of epithelioid cell nodules which do not undergo necrosis or caseation.

The avian type is the least virulent and the bacilli, though they may multiply in the organs and glands (in Hamster 6 profusely, causing death) rarely give rise to macroscopic tuberculous lesions.

REFERENCE

GRIFFITH, A. S. (1939). The relative susceptibility of the field-vole to the bovine, human and avian types of tubercle bacilli and to the vole strain of acid-fast bacillus (Wells, 1937). *J. Hyg., Camb.*, **39**, 244–59.

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