

THE HISTORY OF SIX OLD CULTURES OF  
*MYCOBACTERIUM TUBERCULOSIS*

BY THE LATE A. STANLEY GRIFFITH, C.B.E., M.D., PH.D.

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INTRODUCTION

INSTANCES are recorded in the literature of mammalian tubercle bacilli having lost their virulence completely during long cultivation on artificial media. In a paper published in 1925 I gave an account of the results of investigating two such strains which had been deposited in the National Collection of Type Cultures by Dr Nathan Raw. These strains were stated by Dr Raw to be lineal descendants of strains which had been given to him in 1906, one (human) by Prof. R. Koch, who had isolated it from the sputum of a case of human pulmonary tuberculosis, the other (bovine) by Prof. A. Calmette, who had obtained it from a mesenteric gland of a cow.

Dr Raw (1921) stated that the strains had been subcultivated every month on glycerine agar, and that every year since 1906 they had been injected into animals. No change was noticed in the virulence of the strains down to the year 1912. Details were, however, not given of these virulence tests. In 1913 and subsequent years until 1919 the cultures were frequently tested—on rabbits and guinea-pigs—and were found to be non-pathogenic on every occasion.

In 1921 I obtained the two strains from the National Collection and found that they produced on artificial media, instead of the dry coherent growths of the mammalian types, slimy growths similar to those of bacilli of avian type. The strains were therefore submitted to the various tests which serve to distinguish the avian type from the two mammalian types. The strains were not

virulent for the fowl but reacted in other respects like tubercle bacilli of avian type, that is to say, they were pleomorphic, producing in pure cultures on egg filamentous, clubbed and branched forms; absorbed the agglutinin from avian sera, were not virulent for the guinea-pig but caused disease of Yersin type in this species when inoculated intraperitoneally in large doses and set up joint tuberculosis in rabbits inoculated intravenously.

The results in rabbits were important, and are summarized. Six rabbits were inoculated intravenously with each strain, and five in each group showed tuberculosis of the joints 5 months or more later. One rabbit in the Calmette series, no. 2728, which died 468 days after inoculation, showed, besides joint disease, three tubercles and a caseous nodule in the lungs from which a culture of human type was obtained. The almost unfailing regularity with which these strains produced tuberculosis of the joints in rabbits was very striking and suggested the importance of trying to raise the virulence of the strains for the fowl by passage through the bodies of rabbits and in this way confirming the diagnosis of type based on the other differential characteristics. Accordingly, each strain was passed by intravenous inoculation through several rabbits in succession, the culture being recovered from a tuberculous joint of each rabbit (except R. 2991 which died acutely) in the passage. These passage experiments were not completed in 1925, and the results are now put on record together with those of investigating four other strains which also did not correspond in their characters to their label descriptions.

#### ORIGIN OF STRAINS INVESTIGATED

Designation of strain	Derivation of original culture and source of strain investigated
(1) Koch-Raw	Human strain originally isolated by Koch from the sputum of an advanced case of pulmonary tuberculosis; given to Dr Nathan Raw in 1906 and presented by him in 1921 to the National Collection of Type Cultures; obtained by me in 1921 from the Collection
(2) Calmette-Raw	Bovine strain from a mesenteric gland of a cow; given to Dr Nathan Raw by Prof. A. Calmette in 1906; presented to the National Collection in 1921, in which year I obtained a subculture of the strain
(3) Pasteur-bovine	Supposed to have been derived from a ?horse; received in Manchester in 1902 from the Pasteur Institute, Paris. A subculture was sent to me in 1924 by Dr G. S. Wilson
(4) Spahlinger-Gloyne	Derivation unknown; received by Dr S. Roodhouse Gloyne in 1913 from Spahlinger
(5) Arloing-Courmont	Human bacilli made to grow homogeneously in broth at temperatures up to 44° C.; sent by Dr G. S. Wilson in 1904
(6) Gladys Lyons	The Gladys Lyons strain was isolated by A. S. Griffith in 1918 from the sputum of a girl. The strain passed through several laboratories and was returned to the Field Laboratories by Dr R. G. Canti in 1925

## (1) THE KOCH-RAW STRAIN

*Passage experiments on the rabbit*

Passage rabbit	Source of culture	Method of inoculation	Dose mg.	Duration of life days	Result
2804	Original	Intrav.	10.0	K. 154	Tuberculosis of right tarsal, left shoulder and left elbow-joints
2938	R. 2804 (foot)	Intrav.	10.0	D. 168	Tuberculosis of both wrist-joints
2991	R. 2938 (wrist)	Intrav.	5.0	D. 24	Congestion and oedema of lungs. Large soft red spleen. Marked anaemia. Yersin type of disease
3067	R. 2991 (spleen)	Intrav.	10.0	K. 287	Tuberculosis of left knee- and elbow-joints. A few grey white points in the lungs, ? healed tubercles
3191	R. 3067 (knee)	Intrav.	5.0	K. 88 (paralysis of hind-limbs)	Tuberculosis of both knee- and shoulder-joints and a sacro-iliac joint. Caseo-pus in two vertebrae (12th dorsal and 1st lumbar) and at level of latter a thin-walled caseous abscess, size of a millet seed, projected into the canal (cause of the paralysis)
3219	R. 3191 (shoulder)	Intrav.	5.0	D. 263	Tuberculosis of both knee-joints, both wrist-joints, eight metatarsal and several metacarpal joints, the left shoulder-joint, the tendon sheaths on the dorsum of the left foot and the bursa of each tendo achillis

*Virulence tests on fowls with the Koch-Raw strain recovered from rabbits*

Fowl no.	Source of culture	Method of inoculation	Dose mg.	Duration of life days	Result
36	R. 3067 (knee)	Intrav.	10.0	K. 74	No sign of tuberculosis. Killed by companions
37	do.	Intrav.	10.0	K. 205	Scattered tubercles in liver, grey or caseous centrally with fibrous margins. Three caseous tubercles in spleen (fully virulent avian bacilli from spleen)
40	R. 3191 (shoulder)	Intramusc.	20.0	K. 273	Local lesion only; culture from spleen sterile

After passage through the bodies of four rabbits (the last rabbit 3067) in succession the Koch-Raw strain was tested on two fowls, each intravenously with 10.0 mg. One fowl was killed by its companions 74 days after inoculation and showed no sign of tuberculosis. The other was killed 205 days after inoculation and showed three caseous tubercles in the spleen and scattered grey fibrous tubercles, some centrally caseous, in the liver. A culture obtained from the spleen was avian culturally and highly virulent for two fowls which were inoculated intramuscularly each with 20 mg. of the culture and died of acute tuberculosis in 26 and 32 days.

The strain from rabbit 3067 was tested on rabbit 3191 and recovered from the shoulder-joint of the latter. Inoculated intramuscularly in a dose of

20.0 mg. the culture produced a local lesion only in fowl 40 (killed, 273 days); cultures from the spleen were sterile.

After residing 721 days in the bodies of five rabbits, all of which became infected, four with joint tuberculosis, the strain did not acquire virulence for the fowl, or become increased in virulence for the rabbit. There is little doubt therefore that fowl 37 which yielded a virulent culture of avian tubercle bacilli was spontaneously infected.

## (2) THE CALMETTE-RAW STRAIN

### *Passage experiments on the rabbit*

Passage rabbit	Source of culture	Method of inoculation	Dose mg.	Duration of life days	Result
2865	Original	Intrav.	2.5	K. 235	Tuberculosis of right elbow-joint and bursa and of a right metacarpal joint
2952	R. 2865 (elbow)	Intrav.	10.0	K. 293	Slight tuberculosis of a tendon sheath over right shoulder-joint
3063	R. 2952 (shoulder)	Intrav.	10.0	K. 281	There was no sign of disease internally. The following joints were tuberculous. left elbow, right carpus, right ankle and a few metatarsal; also bursa of left tendo achillis
3182	R. 3063 (bursa)	Intrav.	10.0	D. 602	Tuberculosis of both carpal and knee-joints, latter dislocated, one small grey nodule in lungs
3309	R. 3182 (carpus)	Intrav.	5.0	K. 607	No sign of tuberculosis
3183	R. 3063 (bursa)	Intrav.	5.0	K. 213	Tuberculosis of one metatarsal joint
3248	R. 3183 (foot)	Intrav.	10.0	D. 664	Tuberculosis of left knee-joint, sacculus and vermiform, some minute tubercles on mesentery and in mesenteric glands

### *Virulence tests on fowls with the Calmette-Raw strain recovered from rabbits*

Fowl no.	Source of culture	Method of inoculation	Dose mg.	Duration of life days	Result
34*	R. 3063 (tendo achillis)	Intrav.	10.0	K. 203	No sign of illness. Two moist colonies from spleen
35	do.	Subcut. and intramusc.	20.0	K. 203	Local lesions only. Cultures recovered from lesion, not from spleen
41	R. 3183 (foot)	Intrav.	10.0	K. 265	No sign of disease. Cultures sterile
42	do.	Intramusc.	30.0	K. 259	No sign of disease. Cultures sterile

\* A single colony strain from fowl 34 was inoculated intravenously (dose 10.0 mg.) into two fowls. They were killed 258 and 278 days after inoculation and showed no tuberculosis. A single moist colony grew on one of two tubes sown with spleen tissue from the latter.

The Calmette-Raw strain was passed through five rabbits in succession and was recovered in culture from a tuberculous joint in each passage rabbit. The culture from rabbit 3063 was tested on two rabbits (3182 and 3183) both of which developed joint disease. A culture obtained from rabbit 3182 did not infect a rabbit into which it was injected intravenously. The third and fourth rabbit passage cultures (809 and 1022 days' residence respectively) were each tested on two fowls which when killed showed no sign of tuberculosis. The virulence of the strain for the fowl was not enhanced by passage through the rabbit.

The cultural characteristics of all the strains recovered from animals were identical with those of the original strain.

### (3) THE PASTEUR-BOVINE STRAIN

This strain exhibited the cultural characteristics and serological reactions of the avian type of tubercle bacillus. Microscopically branched forms were infrequent; in some smears, however, club-shaped forms were numerous. The strain was not virulent for the guinea-pig or the fowl but produced joint disease in rabbits inoculated intravenously.

#### *Inoculation experiments on rabbits and fowls with the Pasteur-bovine strain*

All the injections were intravenous, except fowl 31

No. of animal	Source of culture	Dose mg.	Duration of life days	Result
R. 3101	Original	10-20	K. 222	Tuberculosis of knees, ankles, wrists and shoulders and of right heel (bursa); in connexion with the shoulders and the heel there were very large thin-walled abscesses; T b. were very numerous in the pus
R. 3166	R. 3101 (shoulder)	10.0	K. 128 (paralysed)	Tuberculosis of both knee- and one shoulder-joint. Pea-sized collection of caseo-pus between spines of 11th and 12th vertebrae
R. 3167	do.	10.0	D. 80	Two minute tubercles in lungs. Tuberculosis of right knee-joint
R. 3217	R. 3166 (knee)	5.0	D. 252	Almost all the limb joints were tuberculous. The left hip was extensively disorganized and the femur was dislocated backwards on to the sacrum, the head of the bone having disappeared. The right hip also showed advanced disease. The left knee-joint was dislocated and the left tibia showed a spontaneous fracture, the medullary canal containing soft red granulation tissue swarming with tubercle bacilli. T b. were numerous in all the lesions
F. 30	R. 3101 (shoulder)	10.0	K. 219	No sign of disease. One colony from the spleen
F. 31	do.	15.0 (intramusc.)	K. 219	Local necrotic lesion; no disease elsewhere. Moderate number of colonies from the lesion, none from spleen
F. 45	F. 30 (spleen)	10.0	K. 258	No sign of disease. Cultures from spleen negative
F. 46	do.	10.0	D. 25	Numerous grey barely visible points in liver, no sign of disease elsewhere. Avian type cultures from liver

## (4) THE SPAHLINGER-GLOYNE STRAIN

In 1913 Spahlinger gave to Dr S. R. Gloyne a batch of tubercle cultures. During the period of the war the strains were not subcultured and all became overgrown with moulds or died save one, a subculture of which was sent to me by Dr Gloyne in 1925, together with a letter from which I am permitted

*Summary of inoculation experiments on rabbits, guinea-pigs and fowls*

No. of animal	Source of culture	Method of inoculation	Dose mg.	Duration of life days	Result
R. 3133	Original	Intrav.	10 0	K. 151 (paralysed)	Tuberculosis of left knee-joint, not severe; T b in moderate number. No disease elsewhere
R. 3134	do.	Intrav.	10.0	D. 15	Tuberculosis of Yersin type
R. 3194	R. 3133 (knee)	Intrav.	2 0	D. 23	Congested lungs and enlarged spleen. Cultures from spleen produced an abundant out-crop of colonies on fragments of splenic tissue
R. 4305	Original	Intrav.	5.0	D. 239	Lungs crepitant; at posterior tip of one caudal lobe half a dozen caseous miliary tubercles; otherwise normal. The right knee, left elbow, left ankle, both wrists, several small joints of carpus and tarsus were tuberculous and contained pus; no lesions were found in the marrow of the knee epiphysis or long bones of femur. Several subperiosteal abscesses of bones of legs, the bone beneath being a little roughened. Microscopically; right knee, T.b + + +, left elbow, T.b. + +
G.P. 4486	Original	Intrap.	5.0	K. 217	Small abscess in omentum. No disease elsewhere. Cultures from omentum negative, scattered colonies from spleen
G.P. 4487	do.	Subcut.	10.0	K. 217	No sign of disease. Cultures from inguinal gland negative
G.P. 7108	do.	Intrap.	5 0	D. 14	Omentum near pylorus slightly thickened, contained one pea-sized softened nodule; a similar one on parietal peritoneum. No sign of tuberculosis in organs. Microscopically, a few T.b in spleen
G.P. 7109	do.	Subcut	5.0	K. 443	No sign of tuberculosis. Inguinal glands normal and no local scar
F. 220	do.	Intrav.	5 0	K. 158	No sign of tuberculosis
F. 167	do.	Intrav.	1.0	D. 410	No sign of tuberculosis
F. 168	do.	Intrav.	1 0	D. 19	Some doubtful nodules in liver, not recent
F. 169	do.	Intramusc.	22 0	D. 167	Dry cheesy encapsulated mass in pectoral muscles

to quote the following passages: 'I came to the conclusion at the time that this was a strain of avian tubercle of very low virulence, although Spahlinger had apparently not recognized it and I should not be surprised if it were a subculture from the original Koch or Calmette strain, of which subcultures also got into the hands of Nathan Raw. Another interesting point is that Spahlinger demonstrated in 1913 some guinea-pigs which he had infected with tubercle bacilli and then cured with his vaccine serum treatment. I have always felt that it was quite possible that the guinea-pigs were infected with this avian strain which may account for his having cured them.'

On glycerin egg the culture produced good cream-coloured growth which did not spread so easily as a typical avian strain. On glycerin agar the growths were dry, greyish white, ground glass layers which varied in thickness and were finely wrinkled with granular surfaces. On glycerin potato the layers were fairly thick, cream-coloured and warty. The growths on agar and potato were coherent and could be lifted from the media *en masse*. In primary cultures on egg and glycerinated potato when the colonies were few they were raised granular and warty with thin grey ground glass margins which in some instances spread on to the walls of the tube. When the primary colonies were numerous on egg secondary white colonies developed and these were occasionally umbilicated. When fragments of tissue containing numerous tubercle bacilli were left on the surface colonies grew profusely from them. The bacilli in the white colonies were pleomorphic and included some irregular streptothrix and a few club-shaped forms. No typical branched organisms were seen, but there were occasional T- or Y-shaped forms.

#### (5) THE ARLOING-COURMONT STRAIN

##### *Inoculation experiments*

The injections were intravenous, except G P. 4532

No. of animal	Source of culture	Dose mg.	Duration of life days	Result
R. 3100	Original	10 0	K 223	Moderate number grey foci in liver. A few submiliary tubercles, some calcareous, in lungs. Early tuberculosis of left elbow (T.b. scanty)
R. 3168	R 3100 (elbow)	10 0	D. 19	Lungs congested and oedematous. Spleen large and red. Excess of fluid in abdominal cavity
R. 3169	do.	10 0	D. 15	
R. 3185	do	5 0	D 25	
R. 3220	R. 3168	10 0	D. 15	Lungs oedematous, contained scattered tubercles. Spleen enlarged. Fluid in abdominal cavity
G.P. 4532	R 3100 (elbow)	5 0 (intrap.)	K. 212	Omentum contained small abscesses. Manual glands softened. Cultures from omentum and liver negative
F. 32	do.	10 0	K. 206	No sign of disease. Cultures from spleen negative
F. 33	do.	10 0	K. 226	No sign of disease. Cultures from spleen negative

This strain exhibited the morphology, cultural characteristics and serological reactions of the avian type of tubercle bacillus. The virulence of the strain was tested on the rabbit, the guinea-pig and the fowl. The strain was not virulent for the guinea-pig or the fowl but produced joint disease in one rabbit. Four rabbits inoculated with joint strain died prematurely.

#### (6) THE GLADYS LYONS STRAIN

This was an example of change of characteristics in one of my own strains which, however, did not occur in my laboratory. In 1918 I obtained a culture directly from the sputum of a girl. A subculture of this strain was sent to a colleague who used it for complement-fixation tests. As the strain proved very suitable for this purpose it was passed on to other laboratories. Through how many hands the culture passed I do not know, but in 1925 an alleged subculture was sent to me by Dr R. G. Canti with the information that it had undergone a change in its characteristics.

The original strain, which had been maintained in my laboratory, remained a typical human strain alike in cultural characters and in virulence. The strain which was sent to me, labelled 'Gladys Lyons', formed moist whitish growths on media and in its cultural characteristics and morphology resembled a strain of avian type. The culture partially absorbed the agglutinin from an avian serum. It proved non-virulent for guinea-pigs, fowls, sparrows, and toads (T.), but produced joint disease in one rabbit inoculated intravenously.

#### *Inoculation experiments on animals with Canti's Gladys Lyons strain*

No. of animal	Source of culture	Method of inoculation	Dose mg.	Duration of life days	Result
R. 3164	Original	Intrav.	10.0	D. 24	Lungs oedematous Liver and spleen speckled grey foci. A few tubercles in areolar tissues. Cultures of avian type recovered
R. 3178	do.	do.	5.0	D. 24	Lungs, scattered tubercles. Spleen, large, red and speckled General anaemia
R. 3179	do.	do.	5.0	D. 26	do.
R. 3186	R. 3164 (spleen)	do.	5.0	D. 15	Tuberculosis of Yersin type
R. 3195	R. 3186 (spleen)	do.	1.0	K. 404	Tuberculosis of right knee-joint. Slight enlargement of the left carpus but no pus. Lungs contained three hemp-seed-sized caseous nodules and scattered miliary tubercles. Scattered typical bovine nodules in kidneys Cultures of avian type were obtained from the knee and of bovine type from the kidney



*Inoculation experiments on animals with Canti's Gladys Lyons strain*  
(continued)

No. of animal	Source of culture	Method of inoculation	Dose mg.	Duration of life days	Result
R. 3208	Original	Intrav.	10 0	D. 841	Small abscess near one metacarpal joint containing small white bodies like seeds and T.b. in moderate number Cultures failed
R. 3209	do.	do.	5 0	K. 59	Doubtful foci in lungs. Scattered opaque foci in liver. Minute pits on surface of each kidney. Avian cultures from liver and spleen
G. P. 4478	do.	Subcut.	10.0	K. 214	No sign of tuberculosis Cultures from inguinal glands negative
G. P. 4524	do.	Intrap.	10 0	D. 36	Several softened nodules in omentum Sternal and pyloric glands softened Spleen slightly enlarged. Some fibrinous deposit on peritoneum Cultures of avian type recovered
G.P. 4688	do	do.	10 0	K. 268	Three softened nodules in omentum and one in abdominal wall. No tuberculosis elsewhere. Cultures negative
G.P. 4689	do.	do.	20 0	D. 10	Acute tuberculosis. Avian cultures recovered
G.P. 4939	R. 3195 (knee)	Subcut.	5.0	D. 136	Softened nodule in each inguinal gland and no sign of tuberculosis elsewhere. A single avian colony grew on one glycerine egg tube sown with gland pus
G.P. 4940	R. 3195 (kidney)	do.	Small	D. 54	Moderate generalized tuberculosis (mammalian)
F. 48	Original	Intrav.	10.0	D. 30	Spleen enlarged. Liver enlarged and pale, appeared under magnification to be peppered with small grey foci. Numerous avian colonies obtained from liver and spleen
F. 49	do.	Intramusc.	20 0	K. 268	Small local lesion only. Cultures from spleen negative
T. 72	do.	Subcut.	Large	K. 503	No sign of disease. A few clumps of T.b. seen in a smear from liver, which yielded discrete avian colonies
T. 73	do.	do.	do.	D. 889	No sign of disease. Numerous T.b. found in smear of liver, an emulsion of which yielded twenty avian colonies on each of two egg tubes
T. 74	do.	do.	do.	D. 917	No sign of disease. A smear preparation of liver showed clumps or masses of T.b. but cultures from liver yielded only nine avian colonies on one tube and twenty on another

## DISCUSSION

Six strains of tubercle bacilli, all of which were believed to be descendants of mammalian strains originally isolated from mammalian—human, bovine or equine—tuberculous lesions, were obtained for investigation because they were stated to have lost their virulence completely for animals.

As in my experience up to 1925 the characteristic virulence of mammalian tubercle bacilli is not lost (three instances have since been recorded by me) during prolonged subcultivation on artificial media I obtained these strains to ascertain whether they had retained any character by which they might still respectively be identified with the type to which they were stated originally to have belonged or whether there had been a replacement by tubercle bacilli of another type.

The strains were therefore submitted to the various tests which serve to distinguish the mammalian types from each other and from the avian type. The results are summarized in four sections dealing respectively with cultural characters, morphology, pathogenicity, serological reactions.

*Cultural characters.* Five of the strains produced, instead of the dry crumbly and coherent growths of old cultures of mammalian tubercle bacilli, the slimy, easily emulsifiable growths of the avian type of tubercle bacillus. The remaining strain (Spahlinger-Gloyne) produced wrinkled coherent layers resembling superficially those of eugonic human strains but of a softer, more emulsifiable nature than eugonic human growths. When fragments of the tuberculous tissues were placed upon the medium these become covered with outcrops of small waxy white soft colonies. Mammalian strains may grow in this way, but not so regularly or so profusely, and form greyish white granular colonies.

*Morphological characters.* In cultures avian tubercle bacilli exhibit greater pleomorphism than mammalian tubercle bacilli, especially when growing in primary culture on plain egg. If an emulsion of avian tuberculous material is sown on to the surface of an egg tube, there will be seen within 14 days minute flat transparent colonies. On further incubation some of the colonies will become raised, opaque, ivory-white and rounded, umbilicated or crateriform with smooth waxy surfaces.

Microscopically the elements of the 'white' colonies show great variety of form. Stained in the ordinary way for tubercle bacilli many of the organisms are not acid-fast and are coloured blue. These blue-stained organisms are granular and may resemble streptococcal chains. The acid-fast elements are short bacilli, long beaded streptothrix and clubbed forms and branched filaments.

A quick way of detecting branched forms is to take an umbilicated colony and break it down in a drop of salt solution with a platinum spatula. In the turbid suspension thus formed fine shreds may be seen which are composed of streptothrix and branched forms.

Each of the six strains was recovered in culture on plain egg from animals

which had been inoculated to test the virulence. The umbilicated white colonies which each strain produced in primary culture on egg exhibited the pleomorphism of the avian type. A varying proportion of the organisms were faintly stained blue and were either granular, resembling streptococcal chains, or amorphous. The acid-fast bacilli were mainly short; though some were filamentous, clubbed or branched. True branched forms were easily found in the primary umbilicated colonies produced by the Koch-Raw, Arlong-Courmont and Gladys Lyons strains and were infrequent in the Calmette-Raw, Pasteur-bovine and Spahlinger-Gloyne strains. In the colonies of the last three strains an occasional T- or Y-shaped form was seen but on the other hand filamentous or club-shaped forms were sometimes numerous.

*Pathogenicity.* All the strains were tested on fowls, guinea-pigs and rabbits and one strain was tested on toads.

None of the strains was pathogenic for the fowl, inoculated intravenously or intramuscularly, or for the guinea-pig inoculated subcutaneously. Some of the strains inoculated intraperitoneally in relatively large dose in guinea-pigs (they were not all so tested) produced disease of Yersin type.

Next to the fowl and guinea-pig the rabbit is the most useful animal for differentiating avian from mammalian tubercle bacilli. The rabbit is very susceptible to infection with avian tubercle bacilli, and develops generalized tuberculosis after intravenous inoculation of extremely small doses of culture. The disease has a characteristic distribution, an almost invariable feature in chronic infections being tuberculosis of the joints.

Each of the strains was injected intravenously into rabbits, one or more of which developed tuberculosis of a joint or several joints, from which cultures identical with the original strain were recovered.

Two of the rabbits (nos. 2728 and 3195) showed lesions internally from which mammalian cultures were obtained, rabbit 2728 injected with the Calmette-Raw strain yielding a human culture and rabbit 3195 injected with the Gladys Lyons strain a bovine culture. It is interesting that these strains failed to raise the resistance of rabbits to spontaneous infection with mammalian tubercle bacilli. With these exceptions the lesions attributable to the strains under test were confined to the joints, bursae, tendon sheaths and bones. I gave descriptions of such joint lesions in 1925. To these may be added accounts of some features of interest in the localization and effects of the lesions.

Two rabbits became paralysed in the hind-limbs; the paralysis in each case was found to be due to the pressure on the cord of an abscess originating in the spinal column. In two rabbits (3182 and 3217) the bones of the legs were weakened by caseo-necrosis of the marrow and had fractured, resulting in dislocation of knees and hips. In one the bone lesions were subperiosteal.

It was noteworthy that the eyes and the lachrymal glands, organs which are not affected in rabbits inoculated with fully virulent avian strains, but frequently affected in rabbits suffering from chronic infections with mam-

malian strains, showed no evidence of tuberculosis in any of these chronically affected rabbits.

The experiments on the rabbit proved conclusively that all the six strains were pathogenic and capable of producing active tuberculosis in this species of animal. The localization of the lesions in the joints of the rabbits and the regularity with which these structures became affected strongly support the view that the strains were of avian type.

Three toads were injected subcutaneously under the skin of the back each with a large unmeasured dose of a 5-day-old glycerine-egg culture of Canti's Gladys Lyons strain. The experiments were made to ascertain the virulence of the strain for toads and, if not pathogenic, how long a strain of avian type would remain viable in the tissues of the toad. The toads were examined 503, 889 and 917 days after inoculation and were free from macroscopic lesions. Avian bacilli were cultivated from the liver in each case. The number of colonies, however, obtained from the liver of the last toad was disproportionately small to the number seen in a smear preparation and seemed to indicate that many of the bacilli stored up in this organ were dead (see also Griffith, 1941).

*Serological reactions.* The Koch-Raw, Calmette-Raw, Arloing-Courmont, Pasteur-bovine and Gladys Lyons strains absorbed the agglutinin completely from avian sera. The Spahlinger-Gloyne strain did not absorb the specific agglutinin.

#### SUMMARY

1. Six old laboratory strains of tubercle bacilli, supposedly of mammalian origin, have been investigated.
2. Five strains produced on artificial media the soft, easily emulsifiable, growths which are characteristic of the avian type of tubercle bacillus.
3. The sixth strain formed in subcultures coherent, wrinkled, layers superficially resembling those of eugonic human strains but of a more easily emulsifiable nature.
4. All the strains were pleomorphic, the primary white colonies on egg showing streptothrix, bulbous or club-shaped and branched forms. The branched forms were easily found and typical in the white colonies of the Koch-Raw, Arloing-Courmont and Gladys Lyons strains; they were infrequent and usually T- or Y-shaped in those of the Calmette-Raw, Pasteur-bovine and Spahlinger-Gloyne strains.
5. None of the strains was virulent for the guinea-pig or fowl. They all, however, in rabbits inoculated intravenously, gave rise to joint disease similar to that produced by typical avian tubercle bacilli.
6. The five avian strains with typical avian cultural characters absorbed the specific agglutinins from avian sera. The Spahlinger-Gloyne strain, which was not soft and slimy in cultures, did not absorb the agglutinins from avian sera.

[*Conclusions.* Dr Griffith left only some rough notes relating to the conclusions. From these and from conversations it is clear that he did not regard the apparent changes in type as due to true modification. He appeared to think that in some cases the apparent change might have been due to accidental contamination with an avian strain during subcultivation, and possibly in others to impurity owing to double infection in the original culture, the avian strain alone surviving (see Agricultural Research Council's Committee, 1941, p. 316).—ED.]

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