completion of thoracoplasty. Similar observations of benefit from thoracoplasty were made in cases with long-standing empyema or with fibrothorax or thickening pleurisy.

Phrenic interruption.—Although the diaphragmatic paralysis frequently produces striking anatomical improvement and is easy to do and causes little or no operative shock, it gives the worst functional results of all collapse procedures. Cournand and Richards found impairment of function evidenced by low ratio of breathing reserve to maximum breathing capacity at rest, and also oxygen unsaturation of arterial blood at rest or after exercise. There is a distinct tendency to pulmonary congestion. Phrenico-paralysed cases are seen, indeed, to become more rapidly dyspnœic at the least exertion than others with bilateral pneumothorax or extensive thoracoplasty.

Extrapleural pneumothorax (Graf, Schmidt).—If for any reason ordinary AP treatment is not practicable, extrapleural pneumothorax is the least mutilating and most elastic operation in collapse therapy. The idea behind the introduction and development of this operation has been to produce the best collapse with the least functional impairment. Functional examinations show that this object has largely been attained. According to Gaubatz, the functional impairment seen after extrapleural pneumolysis is remarkably less than, sometimes only half, that seen after apical or other partial thoracoplasties of corresponding extent.

In estimating immediate surgical risk and ultimate physical capacity, any dysfunction on exercise is much more important than at rest. As the present discussion shows, such dysfunctions happen if the breathing reserve is reduced by extensive tuberculous lesions or by excessive emphysema in long-standing fibrotic-cavernous diseases. The patient's physiological output in exercise consequently fails much more rapidly than in health, although all signs of insufficiency may be absent at rest. Serious deficiency is present if the maximum breathing capacity on exercise is reduced more than 60 per cent. Again, the time needed for recovering after exercise is much more significant than the immediate effect. In tubercular patients the recovery may last more than 30 minutes compared with 3 to 5 minutes in healthy persons. The duration of recovery depends on the amount of oxygen deficit after exercise and on the time needed to compensate it.

Generally, it may be stated that unsatisfactory results in surgery may be predicted—

(1) in reduced breathing reserve of less than 85 per cent of the maximum breathing capacity;
(2) in arterial oxygen unsaturation at rest or after exercise;
(3) in high peripheral oxygen deficit;
(4) in increased ventilatory equivalent above 3.6; and

(*Concluded at foot of next column*)

AN INVESTIGATION INTO THE INCIDENCE AND TYPE OF TUBERCULOUS INFECTION IN CATTLE AT AMRITSAR WITH SPECIAL REFERENCE TO HUMAN INFECTIONS

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THE idea of bovine infections being transmitted to human beings was first advanced by Theobald Smith (1896) on his finding this type of bacillus in the cervical glands of a child. Searching investigations of this matter have since then been carried out by numerous workers including Robert Koch and the necessary precautions to safeguard the milk were taken in many countries. In India enquiries on similar lines were carried out by Nield (1902), Wilkinson, Joshi and Gloster (1914) and Taylor (1918). The details have been examined by Glen Leston and Soparkar (1917), who concluded that the cattle infection with tubercle bacillus in India was lower than in the west.

Shether (1921) carried out investigations at Muktesar and typed the strains of bacilli isolated from the specimens collected by Taylor at Ferozepur slaughter house, and advanced his view of the low toxicity and virulence of the various strains of tubercle bacilli affecting the

(*Continued from previous column*)

(5) in myocardial damage as revealed by the electrocardiogram.

Conclusion

Diagnosis, prognosis and indications for collapse therapy can be precisely determined by adding functional examinations to our current clinical, biological and radiological investigations. By these examinations, our useful anatomical and biological knowledge will be much increased. The time is near when our diagnosis, hitherto expressed in anatomical and biological terms, will be completed in those of functional capacity.

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cattle in India, the infection being restricted to a few glands, usually calcified. Taylor himself made a systematic examination of over 6,000 selected cattle slaughtered at Ferozepur and recorded about 3.5 per cent being affected with tuberculosis, of which over 2 per cent showed tubercle bacilli in the lesions. Later in 1927 Soparkar, Edward and Krishnamurti studied the problem again, and on experimental results agreed that the low incidence of clinical tuberculosis in Indian cattle is due to their usual mode of open air living, rather than any question of immunity or resistance in them, or the low virulence of the causative bacillus. Recently in 1931, Soparkar stated in an abstract of paper presented by him at the Indian Science Congress held at Nagpur, that out of 1,116 animals examined by him at the Lahore slaughter house, 225 or 22.85 per cent were found to be infected with tuberculosis and tubercle bacilli were detected in the smears of some, exclusive of others found to be infected on animal tests. The cows showed a lower incidence (21.3 per cent) than buffaloes (23.6 per cent to 31 per cent). No information was however given by him with regard to the type of bacilli found.

We made the study of this problem, by trying to find out the type of bacillus responsible for the infection of extra-pulmonary lesions of the local populace. Strains of bacilli were isolated and typed from the variety of pathological materials received from various hospitals in the Punjab. We observed that out of a series of 62 successfully isolated strains of tubercle bacilli, not a single one was found to belong to the bovine type. An investigation on similar lines had been carried out by Ukil (1933), wherein he did not find a single bovine type of bacillus, out of a series of 60 cases of glandular and osteo-articular lesions. Soparkar (1929) mentioned a similar investigation conducted by him, in a paper read by him at the Indian Science Congress held at Madras. He dealt with 65 cases out of which 40 were of cervical adenitis, 8 of axillary gland tuberculosis, and 17 of pulmonary tuberculosis. None of the above cultures obtained was found to be of the bovine type. It was only at the close of his investigation that he discovered a bovine type of bacillus in a child suffering from cervical adenitis, which was the first case described in India.

The failure to find the bovine type of bacillus in lesions expected to yield it, stimulated further interest in the subject and efforts were made to find out what part, if any, was played by bovines in the transmission of infection to human beings by contact with cattle or by the consumption of milk.

It was thus planned to find out first, the amount of infection in bovines, by carrying out a tuberculin survey with tuberculin obtained from the Imperial Veterinary Research Institute at Muktesar. We started with the eastern side of Amritsar city, our chief areas of operation being division 7 and division 9 of the city. All the herd and the individual cattle of these divisions were subjected to the double intradermal test.

Once the positive reactors were detected, it was our aim to investigate them further for the presence of tubercle bacilli in their milk. The individual animals belonging to the herd under test were seen a day prior to the inoculations, and a clinical examination was made of their general health. Notes were always kept for any special signs such as emaciation, cough, diarrhœa, rise in temperature, enlarged glands, swollen udder or any other lesion suggestive of tuberculous disease. It is of interest to note here that mastitis, recognized to be the stage of the disease when tubercle bacilli are passed freely in milk, has been met with in only two cows which, surprisingly however, did not reveal any bacilli in their milk on repeated examinations. In one of these animals, a sero-sanguinous discharge was made to ooze out on squeezing the udder. Cattle have however been seen showing extreme emaciation and enlarged glands and having a chronic cough or a chronic diarrhœa.

The injections were given intradermally in two divided doses of 0.1 c.cm. each, of the concentrated tuberculin, the first or the sensitizing dose being followed by the second or the testing dose of the same quantity after 48 hours.

Technique of injections

A four-inch-square area of the skin, on the side and in about the middle of the neck, was washed with soap and water and shaved preferably a day prior to the inoculations. Recently British workers have preferred to give injections under the skin of the caudal fold to avoid shaving (*Tubercule*, 1938, vol. 8, 347), but we preferred to stick to the side of the neck, on account of greater convenience. The fold of the shaven skin was firmly held in the jaws of a callipers with a sliding scale, to find out the normal thickness of the animal's skin.

On the following day the part was rubbed with an alcoholic disinfectant and pinched up firmly with the left hand. The injection was then given in the tightly drawn layers of the skin. A broad handled dental syringe of 2 c.cm. capacity, with the barrel marked into divisions of 0.5 c.cm., was employed for the purpose. A fine sharp but short needle fixed to the syringe was pushed in the skin parallel to the surface and just when the eye of the needle had gone under the skin 0.1 c.cm. of tuberculin was pushed in, sometimes requiring a good deal of pressure. A bleb-like elevation of the epidermis about the size of a pea was raised, if the injection was properly done. The depth to which the needle should go under the skin depends upon the normal thickness of the latter. Injections accidentally given deeper into the subcutaneous tissue, elicited a very severe local response, which is not significant. The second or the testing dose was given at the same site and in

exactly the same manner as the sensitizing dose, after an interval of 48 hours.

It has been advocated by some writers that the second dose need not be given to the animals showing a marked reaction to the first dose, but in the present series we gave the second dose in every case for the sake of uniformity of the results.

Reading of the results

The measurements of the fold were taken 40 hours after the first and 24 hours after the second dose, the ultimate results being based on the latter reading. Other factors indicating a positive reaction were local heat, tenderness and œdema. These were always found to accompany a reaction producing an increase of more than 10 mm. to the original measurement of the fold. Without these factors being present, the result was not regarded as positive, except when the measurement showed an increase of at least 30 mm. No marked general reactions have been noted, except that the milch cattle showed a definite decrease in the quantity of their output of milk on the day following the injection, a phenomenon with no explanation.

In cases showing a positive reaction, a classification was made based upon the degree of response elicited, in the following manner :—

The difference between the original and the final reading of the fold	Class of reactor
(*a*) Less than 10 mm. ..	Negative
(*b*) Between 10 mm. to 30 mm.	One plus
(*c*) Between 30 mm. to 50 mm.	Two plus
(*d*) Between 50 mm. to 70 mm. or above.	Three plus

In all 1,234 animals were subjected to the test, 282 of which exhibited positive reactions of one degree or another, as follows :—

Plus one reactors	.. 141
Plus two reactors	.. 87
Plus three reactors	.. 54
	282
Negative reactors ..	.. 935
	1,217
Sold during the test ..	.. 17
TOTAL	.. 1,234

It has been further observed that buffaloes showed a higher incidence and a greater intensity of reaction than cows.

So intense has been the response in some of the buffaloes that an increase of more than 120 mm. was obtained to the original measurement of the fold in about three cases.

All the animals that were tested, were further divided into certain age groups, to find out the frequency of infection in different spans of life, which is represented in the following table :—

Age groups in years	Total number tested	Positives	Percentage
One to three years ..	165	24	14.5
Three to six years ..	263	40	15.2
Six to nine years ..	368	98	26.6
Nine to twelve years ..	234	65	27.8
Twelve to fifteen years ..	49	17	34.7
Fifteen to eighteen years	12	5	41.6
Eighteen to above ..	6	3	50.0
Unknown age ..	120	30	25.0
	1,217	282	

It will be noticed that the amount of infection increases with age in cattle as in man, in India, in contrast to that in human beings in which a steady decline is reported after early adult life.*

Problem of milk

After having found a positive reactor, our further aim had been to proceed with the study of its milk. Samples of milk were collected aseptically, and subjected to microscopical, cultural and biological tests, to find out the presence of tubercle bacilli in them.

Method of collection of milk

Four wide-mouthed sterile test-tubes, which had been plugged with sterile cotton, were taken and about 10 to 15 c.cm. of milk from each quarter of the udder milked into each tube. The upper portion of the tube heated on a spirit lamp flame, and the plug of cotton inserted again after having been flamed. The udder and the hands of the milker were thoroughly washed with soap and water before milking. It has been always preferred to have the specimen of the residual milk, known as strippings. After the tubes had been plugged they were placed in a brass holder and carried to the laboratory.

Treatment in the laboratory

Here the tubes of milk were placed in an electric centrifuge and rotated at 2,500 r.p.m. for 5 to 10 minutes, which would separate the milk in three layers.

(*a*) The top layer of the cream, with a few bacteria.

(*b*) The middle layer of clear bluish white fluid.

(*c*) The bottom layer of suspended particles and the majority of bacteria.

Microscopic examination

A little of the top cream was spread on the slide by means of a platinum loop, after which

* Available data in India and elsewhere are not in agreement with the author's statement. As a rule, the incidence of infection increases with age in man, as has also been noticed in cattle.—EDITOR, *I. M. G.*

the cream was separated by a small metal spoon, which had been passed through the flame. The remaining portion of the milk was decanted to about half an inch of the bottom by means of a wide-bored pipette. A drop of the deposit that had been left was transferred to a clean slide by means of a capillary pipette. A new glass slide was then placed at an angle to the drop and a film made by drawing the slide from one end to the other. The slides thus made were allowed to dry in the air for at least half an hour and then fixed, defatted with ether and alcohol, and stained by the Zeihl Neelsen method.

In our series of experiments on 101 specimens of milk, we examined about 500 slides in the above manner, but were unable to find out the particular octahedral cell groups. Under the 1/12 objective the usual finding met with have been the cellular debris, believed to be leucocytic or alveolar in origin. Bacteria that have sometimes been seen, belong to the cocci group in chanis or clumps, or the ærogenous bacilli. Mycobacterium tuberculosis was not found in any of the slides.

Biological testing

Two guinea-pigs were inoculated for each specimen of milk. The deposit from the bottom of the tube was drawn into a sterile syringe and inoculated in the muscles of the right thigh of the pig in quantities of 5 to 7 c.cm. The inguinal glands on the side of inoculation were rubbed a little before and after the inoculation to cause them a little injury, to facilitate the development of infection in them. The animals were then caged and examined at weekly intervals for any signs of developing tuberculosis. The glands on the inoculated side were carefully palpated on each examination, to find out enlargement of tenderness in them. After 3 weeks one of the animals was sacrificed, to study its viscera, for the progress of the disease, the second one being kept for further observation till 6 to 8 weeks and ultimately sacrificed.

About 48 hours before killing an animal, an injection of 10 c.cm. of old tuberculin was made subcutaneously, to find out if there was a reaction, in case the infection had successfully occurred or, in case the death of the animal took place, if the disease was in active progress (Joseph Race).

It has been observed that animals reacting negatively to the inoculation never revealed any lesions when subsequently post-mortemed. Hence in the latter part of this series of experiments, we did not destroy the animals that had reacted negatively to the tuberculin inoculation and had survived it.

In all about 70 animals were subjected to the tests, out of which 8 died of inter-current infections. Forty-six were inoculated and later on autopsied.* Sixteen were inoculated without any reaction being elicited.

Cultural tests

To the deposit in the tubes an equal quantity of sterile solution of 15 per cent hydrochloric acid was added, and the tubes shaken vigorously. A few drops of the sterile litmus solution were also added as an indicator. A flamed cotton plug was then applied to the mouth of the tube, which was then kept at 37°C. in an incubator for a period of not less than half an hour to dissolve the suspended particles and the cellular debris. The tube was then taken out and the product titrated with a sterile solution of 15 per cent NaOH till the neutral point was reached, as indicated by the litmus indicator. About 3 to 5 c.cm. of this product were now pipetted on the slopes of the artificial culture media (Lowenstein's or Petroff's), the tubes of media plugged with flamed cotton and placed in the incubator in a slanting position for a day or two to allow an intimate contact with the inseminated product. After the material had dried the tubes were put in the vertical position and a hole bored through the cotton plug, to allow oxygen to the growing bacillus. An examination of the tubes was made every 5 to 6 days. None of the tubes in this series of experiments showed a growth of tubercle bacillus.

Discussion

From the above it has been observed that, whereas the infection in cattle is quite common, the milk has shown markedly negative results, with regard to the presence of tubercle bacillus in it. The question arises as to why it should be so ? If cases of surgical tuberculosis are quite commonly met with here as in the European countries, as was suggested by Major-General Hutchinson, at the second all-India veterinary conference held at Calcutta, the question arises what can be the actual source of such infection, if the agency of milk so far held to be a chief factor in the causation of the disease is altogether ruled out ? Bradfield (1925) in a paper in the *Indian Medical Gazette* draws attention to this form of disease as met with in Madras, where the surgical form of tuberculosis comprised 2.1 per cent total admissions in 1924. We have been in communication with the veterinary research workers, and wrote to J. R. Haddow and J. F. Shirlaw, of the Imperial Veterinary Research Institute, Muktesar, on the negative findings of our results, and they were kind to let us know in return, that attempts had been made by them to find out tubercle bacilli from the milk of 22 cases of suspected tubercular mastitis during the last four years with unsuccessful results. It therefore seems that the disease in the cattle so far as this country is concerned takes a very mild course, confining itself to a few glands as stated by workers like Soparker (1925), and the infection of the udder, which is likely to contaminate milk, is rarely reached. 'The experiments of the Royal Commission on Tuberculosis show that a dose of 50 mg. of culture of bovine origin given subcutaneously is

*The results of autopsy have not been given.—EDITOR, *I. M. G.*

almost invariably fatal to the English cattle and, with rare exceptions, produces a generalized tuberculosis of a very severe type, which proves rapidly fatal. A certain proportion of Indian calves equally small in size remain well after similar infection, and when autopsied show slight or minimal lesions, indicating that they are possessed of very high power of resistance to tuberculosis as compared to the English calves' (*Indian Journal of Medical Research*, 1923–26, page 765).

This power of resistance in the Indian cattle, accompanied with the open-air mode of living of these animals, serves to restrict the course of the disease. Hence very few cattle reach a stage when they can infect human beings by contact, or when their milk is teeming with tubercle bacilli. It therefore seems that if a case does happen, in whose lesions the presence of bovine type of bacillus is demonstrated, the bacilli behave in a manner entirely 'zoogenic' and not 'anthropogenic' (Kolle and Hetsch, 1934) and hence the infection persists with that particular case, ultimately perishing with the death of the host. Apart from the above, it has been observed that the actual transmission of the disease took place, only if heavily contaminated milk is consumed for a long period of time.

According to Kossel, investigations made from 1905 to 1909 by the Imperial Sanitary Office in Germany show that 151 children and 200 adults had regularly consumed for a longer or shorter period raw milk from cows seen to have tuberculous lesion of the udder. Only two individuals (and they were very young and belonging to different families) were found to have tuberculous glands of a benign form. In both the cases the cow had a very severe form of mastitis involving the whole gland. The other individuals remained quite healthy. It was thus concluded by Weber, who published this account, that infection could only be produced, with repeated ingestion of considerable quantities of bovine bacilli. In New York City Alfred F. Hess followed for three years 18 cases of children who drank milk in which tubercle bacilli were demonstrated, and found that all but one remained free from tuberculous disease (Fishberg).

It therefore appears that the dangers of milk infection have been overestimated by many writers, when considered in the light of the above facts.

It is further reasoned that if animal tuberculosis were causally connected with the origin and spread of human disease, primary tuberculosis of the intestine would, as Koch pointed out, be a much more frequent disease, especially in large towns where tuberculous milk and butter are used in enormous amounts. Referring to the countries where bovine tuberculosis is practically non-existent or where cow's milk is not used in feeding children, *e.g.* Greenland, we find that the morbidity rate especially of the abdominal type of tuberculosis is not less.

Apart from the above, the usual practice of boiling the milk before consumption is an additional safeguard against the disease in this country.

It therefore appears that the cases of surgical tuberculosis are evolved through human infection of man to man by the human type of bacillus.

If further research in the problem be taken up, and strains of tubercle bacilli be isolated and typed from the bovine lesions, there is a likelihood, that the causative type may be found to be the human type of bacillus, that has been unsuccessful in causing a generalized infection.

Summary and conclusions

1. That about 25 per cent of the 1,234 cattle tested were tuberculous as demonstrated by the double intradermal tuberculin test.
2. That the buffaloes reacted rather strongly to the same amount of tuberculin as compared with cows and showed a higher percentage of infection.
3. That the incidence of infection increases with the age of the cattle.
4. That 101 specimens of milk taken from positive reactors were found to be negative for tubercle bacilli, when investigated microscopically, culturally and by animal inoculation tests.
5. That it is not necessary to boil the milk before use to guard against tuberculous infection, though that would be a good preventive against brucella infections and lapses in sanitary precautions.

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