A British Medical Association Lecture ON GALL STONES AND CANCER OF THE GALL BLADDER:

AN EXPERIMENTAL STUDY.*

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(With Special Plate.)

We have succeeded in producing cancer experimentally in animals by the use of various special agents. Fibiger¹ produced it by the nematode *Spiroptera neoplastica*, Bullock and Curtis² by another parasite, the *Cysticercus fasciolaris*, and Yamagiwa and Ichikawa³ by coal tar. In our own laboratory we have produced cancer by coal tar⁴ and tar extracts,⁵ by pitch,⁶ by soot, by arsenic,⁷ by shale oil and industrial oils derived from shale,⁸ by crude petroleum and lubricating oil, and by a tarry substance prepared from isoprene.⁹ Bloch,¹⁰ and, probably before him, Marie, Clunet, and Raulot-Lapointe,¹¹ induced it by means of x rays. In addition to these cancer-producing agents, we have in the human subject others, the relation of which to cancer cannot reasonably be doubted, though they still lack experimental confirmation: the heat of the kangri-pot causing epithelioma of the thighs and abdomen amongst the natives of Kashmir¹²; the betel preparation associated with buccal cancer in Asia¹³; the bilharzial ova inducing vesical and intestinal cancer in Egypt¹⁴ and Japan; and several others less definitely established.

But these known irritants are responsible for a very small proportion of the cancers that occur. They are all of extrinsic origin and entirely foreign to the economy. They act, presumably, not as simple mechanical agents, but in virtue of some peculiar property they possess. Even if we give full scope to our imagination we cannot inculpate any analogous external irritants in the generality of human or animal cancers. If we are to establish the irritation theory of cancer on a broader and surer basis we must search for possible internal irritants. I have therefore been led to the conclusion that substances capable of producing cancer by their prolonged action on the tissues may be elaborated within the body as the result of other pathological processes. In other words, the main mass of carcinogenic agents are endogenous. How these originate we may sometimes guess: of their chemical nature we are at present entirely ignorant. To entertain such a belief as this is legitimate, but to seek to promulgate it without proof is to hinder rather than to help scientific progress. At first I endeavoured to produce cancer by substances which might be considered as intermediate between wholly extrinsic and wholly endogenous. Knowing that cancer of the intestines progressively increases in frequency from the duodenum to the rectum and that putrefactive changes in the bowel contents increase in parallel, I selected at random certain products of intestinal putrefaction, such as skatol, paracresol, tyrosine, and methyl-mercaptan, and introduced these into the lower bowel of mice, rats, guinea-pigs, and rabbits, thrice weekly for many months, but without producing any signs of tumour formation. Of course, there are numerous other putrefactive products that we might use, and we might be fortunate with some of them. Similar use of a tar extract, which proved to be very potent in inducing epithelioma when applied to the skin of mice, failed to produce intestinal cancer in any of these four species of animals. There is in the human subject, however, one outstanding cancer, cancer of the gall bladder, which is closely associated with a particular irritant, and an irritant, moreover, which is entirely endogenous. To this we have directed our investigations.

GALL STONES AND CANCER IN MAN.

It is beyond the scope of this paper to discuss the etiology of gall stones. It was formerly believed that gall stones originated in the gall bladder owing to stasis of the bile and acute inflammations of the viscus, but this is now discounted, and it is becoming more generally accepted that the part played by the gall bladder is quite secondary, or even passive, and that in all probability there is no lesion in that organ preceding the formation of the calculi. Possibly in the majority of cases gall stones give rise to no definite symptoms during the life of the person and are only discovered *post mortem*. The statistics as to the frequency with which they are found vary from 1.5 to 25 per cent., according to the age composition of the hospital patients. Schröder,¹⁵ who analysed von Recklinghausen's autopsy records, found that 12 per cent. of all persons had gall stones; they were rare under the age of 30 (3 per cent.); they were found in 10 per cent. of the cases between the ages of 30 and 60, whilst 25 per cent. of people over 60 years of age had gall stones. They are thus quite common. Cancer of the gall bladder is by no means a rare disease. Kaufmann¹⁶ states that according to the Breslau statistics it forms 5 per cent., and to the Basle statistics 5.19 per cent., of all carcinomata coming to autopsy. Now, in cancer of the gall bladder gall stones are generally present. In 268 cases collected by Futterer and Haberfeld they were present in 70 per cent.; in Musser's series in 69 per cent.; according to the figures of St. George's Hospital (Rolleston) in 75 per cent.; Winton gives 81 per cent., Zenker 85 per cent., Courvoisier 91 per cent., Siegert 95 per cent., and Janowski 100 per cent.¹⁷ As these figures have been compiled from *post-mortem* records we cannot conclude that gall stones were absent unless there was a definite statement to that effect. Musser, however, states that in 3 of his 100 cases calculi could not be found, and several other writers have noted the occasional absence of gall stones. In what proportion of cases of gall stones does cancer of the gall bladder occur? The statistics on the point are surprising. The lowest figure I have found is that given by Candler¹⁸ from asylum practice—namely, 2 in 315—and the highest that by Slade,¹⁹ 30 per cent. Rolleston gives 4.5 per cent., Mayo slightly under 5 per cent., Moynihan 5 per cent., Riedel 7 to 8 per cent., Mayo-Robson 10 per cent., Schröder 14 per cent. Slade's figures (30 per cent.) are certainly much too high: they were based on an exceptional sample; and some of his illustrations would fail to convince a pathologist of the accuracy of the diagnoses. If we put the figure at 5 per cent. we shall probably be safe, but even if we bring it down to Candler's low estimate it will still be a surprisingly high incidence if we consider how low the incidence of cancer is amongst those who are subjected to other cancer-producing irritants. Probably less than 1 in 1,000 of pitch-workers, tar distillers, paraffin-oil refiners, and betel chewers, ever develop their peculiar cancers; though, on the other hand, the early x-ray operators had an extraordinarily high incidence.

Taking all these facts into consideration—that over 10 per cent. of adults have gall stones, that 5 per cent. of these develop cancer of the gall bladder, and that gall stones are present in practically all cases of gall bladder cancer—we have good grounds for believing that cancer of the gall bladder is the result of the irritation set up by the gall stones. On the other hand, some have maintained that the calculi found in cases of carcinoma are the consequence of the carcinoma and not its cause. Such a view cannot lightly be dismissed. If we accept the vesical origin of gall stones we must admit that such a gross lesion of the epithelium as carcinoma will be likely to induce the formation of calculi, either by reason of some exaggerated or pathological secretion of the malignant cells or by the detachment of tissue fragments that may serve to form nuclei for the deposition of cholesterol from the bile. Gall stones, however, are not specially frequent in cases of metastatic nodules in the gall bladder wall. Secondly, gall stones are sometimes absent in gall bladder cancers. To this very important objection it may be replied that in no case can we be sure that gall stones were not present at some previous time; they may have escaped through the bile duct or have

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ulcerated into an adherent portion of bowel. Mr. F. K. Smith of Aberdeen has been kind enough to supply me with notes of a case of carcinoma of the gall bladder which occurred six years after stones had been removed from that organ. Thirdly, simple tumours of the gall bladder are very rare, and, if we bear in mind the frequency with which true malignant tumours are preceded by benign neoplasia in other instances of irritation cancers, we should expect to encounter the preliminary simple tumours much more commonly, especially in *post-mortem* examinations. I have seen two specimens of simple tumours of the gall bladder removed by operation; in neither were gall stones present, nor was there any sign or history pointing to their previous presence. In the few cases of benign tumours of the gall bladder on record stones were absent in half the number. Lastly, we have no convincing examples of cancer being caused by purely mechanical irritations; calculi in other situations, such as the urinary bladder or appendix, are seldom associated with malignant tumour formation. It may be, of course, that the action of the gall stones is not purely mechanical; their chemical composition may be the important factor, and my colleague Dr. H. J. B. Fry has had some interesting experimental results indicating that the action of a weak tissue irritant, insufficient by itself to initiate tumour formation, may be considerably reinforced by cholesterol.

We are thus confronted with a difficulty which cannot be solved by clinical observations and disputations. Are the gall stones the cause of cancer of the gall bladder, or does cancer of the gall bladder bring about the formation of the gall stones? The hazard of experimental investigation was peculiarly alluring, for successful results would not only solve this long-debated question, but would encourage us to pursue, in spite of failures, our quest for endogenous causes of cancers. But the omens were not wholly favourable.

EXPERIMENTS ON ANIMALS.

Hitherto we have had no experimental proof that purely mechanical irritation is capable of inducing tumours. In my own attempts with such irritants I have failed. Bullock and Rohdenburg,²⁰ in criticizing Fibiger's original experiments, maintained that only excessive hyperplasia of the gastric epithelium had been induced by the mechanical irritation of the spiropterae, and they claimed to have produced similar lesions in the rat's stomach by inserting pieces of sponge into the cavity. Their criticism of Fibiger's results, of which they had not fully informed themselves, was unfortunate, but it has always seemed to me that if they had given longer time to their experiments they might have succeeded in producing actual epithelioma. Just before my experiments were commenced, Kazama²¹ claimed to have induced tumours of hollow viscera in a variety of animals by various mechanical and chemical forms of irritation, such as invaginations of mucosa, insertion of sutures, and injections of lanoline and other substances. The English translation of his paper was rather obscure, but one of his tables showed that, amongst other things, he had introduced pebbles into the gall bladder of dogs, rabbits, and guinea-pigs. He used 30 guinea-pigs, which survived the operation from 1 to 116 days, and he claimed to have produced in them the following results: "papillous growths, 6; heterotopic glandular growths, 10; heterotopic or atypical growths, 3; atypical growths, 5; early adenocarcinoma, 3; adenocarcinoma and metastasis, 4," the latter being in the chest wall in 2, in the intestines in 1, and in the liver in 1. Unfortunately this shows that the number of positive results exceeded the number of animals, and the claim seems the less convincing when we notice that at least one of the animals died within a day or two of operation and none survived for four months. In instituting my own experiments I had to face the fact that the gall bladder of mice is much too small for operation, and that rats, which would otherwise have been the animals of choice, do not possess a gall bladder. Rabbits were too large to use in sufficient numbers. Guinea-pigs, which would be ideal in many respects, are peculiar in that they are not naturally affected with cancer, and that we have always failed to induce tumours in them by any of the

various means we have adopted with success in other species of animals. It may even be said that they practically never develop any sort of tumour. When one remembers that hundreds of thousands of guinea-pigs must have been examined throughout the laboratories of the world and yet the only instance of a tumour being recorded was that mentioned by J. A. Murray²²—a transplantable lipoma—the apparent natural immunity of these animals to the causal agent or agents of cancer will appear all the more striking. Nevertheless we employed guinea-pigs in our experiments, and the result has been successful beyond our hopes.

AUTHOR'S EXPERIMENTS ON ANIMALS.

An area over the lower part of the chest and the upper part of the abdomen was epilated by means of sodium sulphide (10 per cent. solution) and rendered aseptic by the application of violet-green solution (0.5 per cent. brilliant-green and 0.5 per cent. methyl-violet in 50 per cent. alcohol). Sterile sheets covering the rest of the animal were clipped on to the edges of the operation area, and the abdomen was opened by an incision running parallel to the lower ribs. The very thin-walled, semi-transparent, spherical gall bladder was readily found and steadied by two small forceps half an inch apart, and a small incision, just sufficient to admit the foreign body, was made with fine scissors. When this was inserted, the cut edges were united by means of a continuous suture of the finest catgut obtainable threaded on a fine needle, and the two ends of the suture were tied together. It will thus be seen that the ridge projecting into the lumen was doubled on its length, and would therefore appear, when viewed from the inside, as more or less of a nodule. It is important to bear this in mind when considering the changes that one might be inclined to attribute to the action of foreign bodies, especially as the displacement and irregularity of the mucosal elements, when seen in microscopic section, will be much exaggerated and will provide a histological picture that may tempt the uncritical to conclude that a small tumour, even of a malignant nature, has been produced. It seemed to me in reading Kazama's communication, and studying the photomicrographic illustrations that appeared in a subsequent paper by him, that this was the criticism that must be passed on his findings, and, indeed, the examination of the conditions in those animals of mine that died within a short time of the operation supported that judgement.

In 25 guinea-pigs I inserted one gall stone, sometimes two, into the gall bladder. These gall stones, roughly about the size of lentils, had been removed four years previously from a patient with cholelithiasis and had been kept in a dry state. They were not sterilized before use. It may be mentioned, in view of Lazarus-Barlow's statement²³ that gall stones associated with gall bladder cancer contain radium while others do not, that I had these calculi examined by our physicist, Mr. C. E. S. Phillips—using methods that would be approved by physicists—but they did not contain any perceptible trace of radium. In 5 cases small, smooth pebbles, sterilized beforehand, were used as the foreign bodies; in another batch of 5, pilules of pitch were inserted. Finally, I injected melted lanoline by means of a hypodermic syringe into the gall bladder of 5 animals, but the puncture wound had to be closed by a stitch. To enable us to identify each animal, coloured charts of the markings of all the guinea-pigs were made at the time of operation.

The experiments in which lanoline had been injected can be dismissed quite briefly as they yielded no positive results. Three of the animals, Nos. 36, 39, and 40, died eleven days after operation, and in all three there was complete desquamation of the lining epithelium with absence of the gland tubules save at their deepest parts and more especially at the site of the needle puncture where a stitch had to be inserted. Here the displacement and irregularity of the tubular epithelium might give rise to a suspicion of early malignancy, but such an appearance is merely temporary and the normal condition is afterwards restored, as could be seen from an examination of the gall bladder of No. 37, which died at the end of five and a half months. (Fig. 1.) The fifth guinea-pig, No. 38, is still alive eighteen months

FIG. 1. Natural size.

FIG. 2. $\times 35$.

FIG. 3. $\times 35$.

FIG. 4. Natural size.

FIG. 5. Natural size.

FIG. 5. $\times 30$.

FIG. 8. $\times 100$.

FIG. 7. Natural size.

FIG. 9. Natural size.

FIG. 10. $\times 30$.

FIG. 11. Natural size.

FIG. 12. $\times 30$.

FIG. 13. Natural size.

FIG. 14. $\times 30$.

FIG. 15. Natural size.

FIG. 16. $\times 100$.

FIG. 17. Natural size.

FIG. 18. Natural size.

FIG. 19 $\times 60$.

FIG. 20. Natural size.

FIG. 21. $\times 75$.

FIG. 22. $\times 40$.

FIG. 23. $\times 50$.

FIG. 24. $\times 75$.

after operation, and when its gall bladder was inspected by exploratory operation exactly a year after the lanoline had been injected it was found to be perfectly normal.

The solid foreign bodies—gall stones, pebbles, and pitch pellets—gave results comparable to each other, and we may consider them together in the order in which the animals died. Two guinea-pigs with gall stones, Nos. 7 and 9, died within a few days of operation, as the result of tearing out their stitches.

[The illustrations of gall bladders with adjacent liver tissue reproduced in the special plate were photographed from the remnants left after providing material for histological purposes, and do not adequately represent the conditions that presented themselves originally to the naked eye. For the photographs and photomicrographs I am much indebted to Dr. D. J. Reid.]

No. 25: Gall stone inserted on January 15th, 1923. Died on January 29th (2 weeks). The lining epithelium and the more superficial portions of the tubules were destroyed. Displacement and irregularity of the gland epithelium in the vicinity of the sutures.

No. 28: Gall stone inserted on January 11th, 1923. Died on February 13th (1 month). The gall bladder was contracted and its opaque walls were irregularly thickened to 2 mm. or more. The superficial and gland epithelium was destroyed, leaving the inter-tubular connective tissue projecting bare into the lumen. (Fig. 2.) Deep in the thickened wall there were well preserved, though irregular, portions of the gland tubules.

No. 31: Pellet of pitch inserted on January 17th, 1923. Died on May 7th (3½ months). The pitch lay in a small smooth cavity in a thick-walled fibrous mass which represented the gall bladder. For the most part the lining membrane had been destroyed, but over one-third of the circumference of the cavity it had been converted into squamous epithelium, and underneath this the muscular layers were intact, and there was no trace of tubules. At other parts of the thickened wall there was considerable and very irregular proliferation of the glandular epithelium amongst new-formed connective tissue, along with disintegration of the muscle bundles. (Fig. 3.)

No. 11: Two gall stones inserted on January 9th, 1923. Died on May 5th (4 months). The gall bladder wall was slightly thickened, rough, and contracted over the calculi. The superficial epithelium had been lost, but was being regenerated from the deeper parts of the tubules, which were actively proliferating.

No. 24: Two gall stones inserted on January 15th, 1923. Died on June 11th (5 months). The gall bladder was enlarged, and on section apparently multilocular, but the walls were not much thickened except in the loculus containing a stone, where they were over 2 mm. in thickness. (Fig. 4.) Sections through the latter part showed much hyperplasia of gland epithelium with numerous cysts. The condition might be described as cystadenoma of doubtful nature. A small hard subcutaneous nodule was found in the chest wall opposite the liver, but on microscopic examination this was seen to be composed of fibrous tissue only.

No. 26: Pebble inserted on January 16th, 1923. Died on July 1st (5½ months). The gall bladder was represented by an irregular, moderately hard, whitish mass adherent to the liver, and, at one part, insensibly blended with it. The liver substance contained numerous white irregular areas, and on the under surface of the liver there was a subcapsular prolongation from the fundus of the gall bladder. (Fig. 5.) On section the mass was solid save for a central cavity occupied by the pebble, on which there was a thin greenish deposit. Microscopic preparations showed adenocarcinoma of the gall bladder, the appearances being repeated in the subcapsular prolongation and in the white areas in the liver substance. (Fig. 6.)

No. 21: Gall stone inserted on January 15th, 1923. Died on July 2nd (5½ months). There was a small bare area of skin over the lower ribs, with indefinite thickening underneath it. The gall bladder, enlarged and hard, was adherent to the chest wall opposite this area. Section through the gall bladder showed it to be thick-walled, loculated, and in direct continuity with a whitish patch underneath the skin. Microscopic examination revealed adenocarcinoma of the gall bladder invading the chest wall and eroding and destroying the rib cartilages. This formed one part of the subcutaneous nodule, but the greater part of it was composed of loose fibrous tissue.

No. 27: Pebble inserted on January 16th, 1923. Died on July 2nd (5½ months). The gall bladder wall was thin for the most part, but was thickened towards the fundus where the pebble lay. Microscopic examination showed an apparently simple hyperplasia of the gland tubules.

No. 1: Gall stone inserted on January 3rd, 1923. Killed on July 3rd (6 months). The thin-walled gall bladder was firmly contracted round the calculus and gave no evidence of neoplasia.

No. 15: Two gall stones inserted on January 11th, 1923. Died on July 8th (6 months). The gall bladder was very small and contracted over the stones. The wall was thin and translucent.

No. 5: Gall stone inserted on January 15th, 1923. Died on July 14th (6 months). The liver was firmly adherent to the lower ribs and ensiform cartilage, and on being separated disclosed a mass of fibrous tissue, which was in direct continuity with the gall bladder. The latter was thick-walled and had a central cavity occupied by the calculus and another smaller empty loculus. (Fig. 7.) Microscopic examination of sections showed very great proliferation of tubular epithelium in the wall of the gall bladder, and also in the fibrous mass. The adenocarcinoma invaded the ribs. (Fig. 8.)

No. 14: Gall stone inserted on January 9th, 1923. Died on July 15th (6 months). The anterior surface of the liver was adherent to the diaphragm and ribs, but these were easily separated and left an extensive white patch on the liver surface. The gall bladder, continuous below with this patch, was represented by a whitish solid mass fading insensibly into liver tissue. (Fig. 9.) Histological examination revealed extensive proliferation of tubular epithelium, the walls of the tubules being generally one cell thick, infiltrating and destroying the liver parenchyma. (Fig. 10.)

No. 32: Pellet of pitch inserted on January 17th, 1923. Died on July 17th (6 months). There was a subcutaneous nodule over the lower ribs, and on the under surface of the liver there was a large irregular bulging whitish mass merging insensibly into the liver substance. (Fig. 17.) On section, this mass was found to be continuous with one end of a shrunken, irregular, thick-walled gall bladder and, on the other side, with the subcutaneous nodule. For the greater part the mass was necrotic, but at its margins it was white and glistening, and evidently spreading into the liver substance. (Fig. 18.) Microscopic examination of the necrotic portion showed dense areas of leucocytes surrounding the remains of tubules, whilst the white marginal portions exhibited masses of dilated and irregular tubules infiltrating and destroying the liver parenchyma almost to its surface. (Fig. 19.) The subcutaneous nodule was composed of young fibrous tissue in the centre of which were indefinite remains, possibly of tubules.

No. 4: Gall stones inserted on January 4th, 1923. Died on August 7th (7 months). The gall bladder was contracted, with thick soft spongy walls, not sharply delimited from the liver. (Fig. 11.) Microscopic examination showed very irregular proliferation of gland tubules, which were not traced into the liver substance, but approached very closely to it. (Fig. 12.)

No. 22: Two gall stones inserted on January 15th, 1923. The guinea-pig remained in apparently good health, and an exploratory operation was performed on January 15th, 1924 (1 year). The animal died two days later from internal haemorrhage resulting from the breaking down of adhesions. The duct end of the gall bladder was thin-walled and dilated, but the fundus was represented by a hard mass buried in the liver substance and containing a single stone, the other stone being in a diverticulum of the cystic duct. (Fig. 13.) Microscopic examination revealed adenocarcinoma of the gall bladder just beginning to infiltrate the liver substance. (Fig. 14.)

No. 13: Gall stone inserted on January 9th, 1923. An exploratory operation was performed on January 9th, 1924, when, on separating adhesions, the gall bladder was found to be small, fairly translucent, and apparently not thickened. The stone was easily palpable. A loop of intestine was torn, and the animal died of peritonitis on January 18th (1 year, 9 days). Section through the liver and adherent gall bladder showed that, though the aspect of the bladder seen at operation was not much altered, the side adherent to the liver was irregularly thickened and a somewhat haemorrhagic prolongation from it penetrated into the liver substance. Growing from this side of the gall bladder was a soft pedunculated mass of tissue projecting into the cavity and filling it save for the part occupied by the stone. (Fig. 15.) On microscopic examination this tumour mass was seen to be composed of a very cellular stroma, embedded in which were narrow irregular tubules. (Fig. 16.) At other parts the tubules were large and irregular, and lined with many layers of tall columnar epithelium. These structures were traced for some distance into a cirrhotic liver.

No. 30: Pebble inserted on January 19th, 1923. Exploratory operation on January 29th, 1924, revealed dense adhesions around a hard mass occupying the position of the gall bladder, and on trying to separate these the liver was torn and the animal died from internal haemorrhage next day (1 year, 10 days). On subsequent dissection the intestines and omentum were found to be firmly adherent to the gall bladder, which on the other side was adherent to the body wall. The gall bladder itself was a hard mass, solid save for the small cavity in which the stone lay, and in this mass there were patches of whiter tissue along with haemorrhagic areas. (Fig. 20.) At some parts the wall was three-eighths of an inch thick. The stone had a thin coating of brown pigment. Microscopically the wall of the gall bladder was represented by excessively and irregularly proliferated tubular epithelium set in areas of dense connective tissue. (Fig. 21.) Within this mass areas of liver cells were found. (Fig. 22.) Part of the epithelium lining the small cavity was converted into squamous epithelium (Fig. 23), and several of the dilated tubules contained deeply staining concretions. The adherent omentum was infiltrated with malignant cells (Fig. 24), but the process could not be traced into the muscles of the abdominal wall.

In addition to these, 11 with gall stones, 2 with pebbles, and 2 with pitch pellets were still alive at the end of fifteen months. All these have undergone exploratory examinations on the anniversary of their first operation, but it was impossible to say how many of them were malignant owing to the restricted examination permitted by adhesions. The two with pebbles had apparently more or less normal gall bladders. In one of the pitch cases the condition was doubtful; and in the other the appearances seemed to exclude malignancy. Of those with gall stones, 3 were considered to be malignant, 3 doubtful, 3 shrunken without signs of growth, and 2 thin-walled, distended, and probably normal. The final reckoning of the success of the experiments must await a later examination of those animals that still remain alive.

DISCUSSION OF RESULTS OF EXPERIMENTS.

It will be seen from the experiments detailed above that the lesions produced in the gall bladder by the solid foreign bodies gradually increase in severity according to the length of time the animals survived. Soon after their introduction there is denudation of the epithelium lining the viscus, followed later by regeneration from the portions of the gland tubules set deeper in the wall. The activity of this tubular epithelium, accompanied by activity of the stroma, becomes more and more marked, the constant trauma exerted by the foreign body, perhaps accentuated by efforts at expulsion, producing repeated destruction of the surface epithelium and repeated replacement from the gland epithelium. In some cases a state of equilibrium is attained, the wall of the gall bladder either being restored to its normal condition with the calculus lying free in the cavity or, owing to the formation of fibrous tissue, contracting down tightly over the stone, when proliferation ceases. In the majority of instances, however, the epithelial activity continues, the tubules growing this way and that through the wall. We have no landmarks to guide us; the muscular bundles do not form, even in the normal gall bladder, an unbroken investment over the glandular layer; and in these experiments the muscle fibres are broken up and often wholly destroyed. We have, therefore, no means of determining when the heterotopy and irregular growth of the tubular epithelium ceases to be translatable as simple hyperplasia and assumes the definite characters of malignant neoplasia, and the difficulty is increased by the fact that frequently, but not invariably, the lining of the proliferating gland tubules, no matter how irregularly or how far they ramify, remains one cell thick, and the nuclei, at all times poor in chromatin, seldom take a deeper stain. It is only when neighbouring tissues are invaded that we have allowed ourselves to consider the process malignant. Short of that there is considerable room for difference of opinion. It is thus impossible to say in how many of our experiments cancer was produced. To my mind, Nos. 26, 21, 5, 14, 32, 22, 13, and 30—all of which showed involvement of adjacent structures—are beyond reasonable doubt malignant. On the other hand, none of the animals that died within four months presented any lesion that should be regarded as more than regenerative hyperplasia, but the process is so gradual and the dividing line so imperceptible that Kazama's claims regarding his experiments, which at first I was inclined to reject, should not be dismissed as groundless, and doubtless he would have obtained indisputable carcinoma in some cases had his animals lived longer.

The feature of these tumours is the invasive character of the growth, and the liver becomes involved by continuity and not by metastatic process. The infiltration is generally confined to the lobe to which the gall bladder has adhered or two adjacent lobes between which it has become wedged, and in no case has the process spread to lobes at a distance. In histological preparations it is not always possible to distinguish a bile ductule from an invading tubule, but the great irregularity of the proliferation in most cases leaves little room for doubt. From the mass in the liver, or more directly from the gall bladder, the diaphragm or abdominal muscles in the vicinity are invaded, and the process in its extension destroys the ribs, forming a perceptible subcutaneous nodule. In two such nodules the striking thing was the amount of young fibrous tissue and the absence or scarcity of epithelial cells within it. This is possibly due to the necrosis and absorption of the malignant cells owing to the poorly developed vascular supply. In No. 32 this necrosis is specially evident in the greater part of the mass in the liver, the living and active tissue being at the periphery. The involvement of the omental fat shows the true invasive character of the growth, and here the cells have entirely lost their tendency to associate in tubule formation. It will be seen that there were no distant metastases in any of the cases. The absence of distant metastases and the spread of the process by mere continuity of tissues are quite usual features in carcinoma of the human gall bladder. The metaplasia of the lining columnar epithelium

into squamous epithelium, a form more suited to withstand mechanical irritations, seen in two of the cases, is a not uncommon finding in chronic cholecystitis and in carcinoma of the gall bladder in the human subject.

No difference could be found between the solid foreign bodies in respect of the lesions they produced: they were equally effective. The fact that pebbles produced the same results as the gall stones shows clearly that the chemical composition of the calculus plays no part in the process. They supply a purely mechanical stimulus to the cells. The fact that sterilized pebbles and sterilized pitch pellets brought about the malignant proliferation just as readily as the unsterilized gall stones demonstrates that the effects of the latter were not due to any living virus they contained; and the fact that lanoline, soft and incapable of causing repeated cell trauma, did not induce exuberant proliferation would seem wholly to negative the suggestion that some mysterious cancer-producing agent was unwittingly introduced from without when the foreign bodies were inserted into the gall bladder. There still remains the assumption that the action of the solid foreign bodies resulted only in the production of a pathological condition of the tissues of such a nature as to allow of the operation of some other causal agent for the cancer. "Cancer"—so the fatuous platitude runs—"never starts in healthy tissues." As in our previous experiments in cancer production so also in this, the evolution of the stages of the lesions is so gradual that it is impossible to find a point at which we could conceive of one causal factor giving place to another of a different order. Here we have a species of animal in which neither a carcinoma of the gall bladder nor a carcinoma of any kind has ever been found in nature, and yet, when we expose a certain tissue to a mechanical irritation to which that tissue has never been previously subjected, carcinoma results. Are we to imagine that the tissues of the guinea-pig are never subjected to irritations of any kind, and that no condition suitable for the hypothetical agent of cancer is ever produced in them naturally? Are we to imagine that this undiscovered agent lurks in each guinea-pig's body and has never before had an opportunity of producing cancer until we introduced solid foreign bodies into a certain viscus? The simplest explanation, and that which will probably appeal to most of us, is that the constant mechanical irritation of the calculi, operating on a suitable tissue, such as the gall bladder proved to be, alone produced the carcinoma.

CONCLUSION.

From these experimental results we may conclude that, in the human subject, gall stones are capable of causing cancer of the gall bladder. Freely admitting that they do so in a comparatively small proportion of cases, we may premise that in some individuals there is a natural resistance to carcinogenic agents, just as there is a natural resistance to pathogenic organisms, and, learning from our previous experiments that similar tumours may be produced by wholly dissimilar agents, we may account for the carcinomata of the gall bladder which are found in the absence of gall stones as being due to some other causal agent or agents not yet identified.

REFERENCES.

- ¹ Fibiger: Det. Kgl. Danske Videnskabskabernes Selskab, *Biologiske Meddelelser*, 1918-1921. ² Bullock and Curtis: *Proc. New York Path. Soc.*, 1920, vol. 20, Nos. 6-8, p. 149. ³ Yamagiwa and Ichikawa: *Mit. med. Fakultät Kgl. Univ. Tokyo*, vol. 19, No. 4, 1918. ⁴ Leitch: *BRITISH MEDICAL JOURNAL*, 1922, ii, p. 1101. ⁵ Kennaway: *Ibid.*, 1924, i, p. 564. ⁶ *Id. ib.*; also Leitch, *Ibid.*, 1923, ii, p. 1. ⁷ Leitch and Kennaway: *Ibid.*, 1922, ii, p. 1107. ⁸ Leitch: *Ibid.*, 1922, ii, p. 1104. ⁹ Kennaway: *Journ. Path. and Bact.*, July, 1924. ¹⁰ Bloch: *Bull. Congrès du Cancer*, Strasbourg, 1923, vol. 2, p. 31. ¹¹ Marie, Clunet, and Raulot-Lapointe: *Bull. assoc. franç. du Cancer*, 1910, vol. 3, p. 484. ¹² Maxwell: *Lancet*, 1879, i, p. 152; Neve: *BRITISH MEDICAL JOURNAL*, 1910, ii, p. 583. ¹³ Fells: *Ibid.*, 1908, i, p. 1357; Bentall, *Ibid.*, 1908, ii, p. 1428; Davis: *Journ. Amer. Med. Assoc.*, February 27th, 1915, p. 711; Spittell: *BRITISH MEDICAL JOURNAL*, 1924, i, p. 158. ¹⁴ Ferguson: *Journ. Path. and Bact.*, vol. 16, 1911, p. 76; Kazama: *Gann*, 1921, vol. 15, p. 22. ¹⁵ Naunyn: *Cholelithiasis*, New Sydenham Soc. Transl., 1896. ¹⁶ Kaufmann: *Lehrb. Spez. Path. Anat.* ¹⁷ Rolleston: *Diseases of Liver, Gall Bladder, and Bile Duct*, London, 1912. ¹⁸ Candler: *Proc. Roy. Soc. Med.*, 1911 (Path. Sect.), p. 97. ¹⁹ Slade: *Lancet*, 1905, i, p. 1059. ²⁰ Bullock and Rohdenburg: *Journ. Cancer Res.*, 1918, vol. 3, p. 227. ²¹ Kazama: *Japan Med. World*, November 15th, 1922, p. 309. ²² Murray: *Journ. Path. and Bact.*, 1916, vol. 20, p. 200. ²³ Lazarus-Barlow: *BRITISH MEDICAL JOURNAL*, 1914, i, p. 1001.