AN EXPERIMENTAL STUDY ON THE PATHOLOGY OF THE BLACK-LEG.

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(Received for publication December 5, 1925.)

INTRODUCTION.

The Black-leg or "Rauschbrand" is an infectious disease of the cattle and other ruminants produced by the traumatic infection of the spores of *Bac. chauvoei* existing in the earth. It has been said that the human being is not susceptible to this bacillus. But the investigations of human "Gasbrand" in recent World's war seem to point the other way. *Kolle and Hetsch, Steinbrueck, H. Conradi and R. Bielng, Fuerth, T. Aschoff, Fickel, Klose, Eug. Fraenkel, L. Aschoff and Klose, Fraenkel and Zeisler*, and others published their observations which seemed to disprove the insusceptibility of human being to this and the allied bacilli.

From my experience in the examination of many black-leg materials, it was suspected that the anatomical changes shown by so-called black-leg cadavers were not produced by only one kind of bacillus, but the similar changes could be produced by several groups of bacilli which could be culturally or serologically differentiated.

For the present research I used several black-leg strains which were identified to *Kitt's* original strain and guinea-pigs as the experimented animals. I compared my findings with the appearances of human gas edema in various references. The following is an abstract from my original paper written in Japanese in the Japanese Journal of Experimental Medicine Vol. VIII, No. 10, 1924.

Clinical Observation.

Leucocytosis due to pseudoeosinophilia in various degrees precedes the local reaction in the site of inoculation. Then as the normal condition is gradually regained, and as the bacilli

increase and the local reaction becomes stronger, the white blood corpuscles more and more decrease. The number of the red blood corpuscles did not change until about the end of the disease. It makes a sudden decrease in agony. There are no appearance or increase of polychromasia, poikilocytosis, and the mother cells of red blood corpuscles such as normoblasts, megaloblasts, etc. A remarkable local reaction is edema, and somewhat localized tumor does not produce crepitation by pressure. The skin of the focal lesion is hyperaemic at the first stage, and becomes greyish red, showing the depilatory surface with red spots, and the swelled skin excreting reddish exsudate at the last stage.

As the general reaction, there are stronger local reaction preceded by fever, and the acceleration of pulse and respiration. Usually appetite remains somewhat longer. No brain symptoms are recognized. The pulse becomes weak and imperceptible at the last stage of the disease.

Morbid Anatomy.

The main pathological change in this disease is in the skeletal muscles. The affected skin is very much edematous, and easy of depilation. The depilated bare skin shows haemorrhage and bloody imbibition in form of darkish red or light red spots. There is bloody gelatinous exsudation in the subcutis. In two layers, inner and outer, of muscles the changes are distinguished. The inner layer, inclusive of the site of inoculation, is darkish crimson and always shows haemorrhage, edema, necrosis and leucocytic infiltration. When a stronger toxin is administered, the translucent grayish gelatinous exsudation is especially remarkable. The outer layer or the peripheral part of the lesion shows at first bloody gelatinous, then serous exsudation, and gradually shades into the healthy part. In any part, there is no remarkable gas bubbles. There is no remarkable change in the lymph nodes of the whole body. But sinusitis and haemorrhage are often recognized in the regional nodes.

No change is noticed in the pleural and peritoneal cavity and organs therein. No splenic tumor, no spongy liver and no foamy blood in the heart are recognized. But in the cortex of adrenals,

naemorrhage of various degrees is always seen. There is no remarkable change in the brain.

The inoculated part always shows the most violent changes. But when that part is scanty in muscles, (e. g. subcutis of frontal region, subcutis of extremities below the knee and hock, etc.), haemorrhage and edema are very slight.

The change in the muscles usually appears continuously from the lesion of the inoculated part. But in the case of intravenous injection, the lesion is not always proportional to the distance from the original spot. For instance, when the injection is made intravenously in the fore arm, there is often a violent change in the thigh which is wide apart from the part of inoculation, and no perceptible change in the intermediate parts, e. g. pectoral and abdominal walls.

Histological Findings.

The most important and ever present change is in the skeletal muscles, adjacent subcutaneous lymph nodes and skin, and then in adrenals. There is no remarkable change in other organs.

1. Muscles.

The change in the muscles may approximately be divided into three stages.

- (i) The first stage or the stage of cloudy swelling and edema formation.

The muscle fibres swell up, and become cloudy, and the transverse striation disappears. In the nuclei of muscle cells, nearly always pycnosis, caryorrhexis and caryolysis, sometimes swelling are recognized. Capillary hyperaemia is seen in the muscle fibres. Haemorrhage and serous infiltration are recognized in the perineural and perivascular loose connective tissues. There is very little fibrin exsudation and no cell infiltration.

- (ii) The second stage or the stage of waxy degeneration.

Fragmentation and fibrillation are seen in the muscle fibers, and the appearance of so-called waxy or *Zenker's* degeneration is manifested. Haemorrhage and sero-fibrinous infiltration are much stronger than in the former stage, and

they extend into the muscle fibres. The cell infiltration is not yet great. The gas produced by bacilli forms bubbles in exsudate, and their rarefaction is found among muscle fibres.

(iii) The third stage or the stage of myolysis.

Myolysis or liquefaction of muscle fibres is noticed in the degenerated muscle fibres. Infiltration becomes richer in cells, pseudoeosinophilic leucocytes, and represents the line of demarkation.

2. *Skin and subcutis.*

Necrosis, edema of the epithelial layer, edema, hyperaemia, haemorrhage and fibrinous thrombi of corium and subcutis are recognized in small veins. The haematogenous pigments are not proved in any part.

3. *Cortical layer of adrenals.*

There is always haemorrhage in the cortical layer of adrenals. The distribution of its lipoid is uneven. Chromaffinity of the medullary layer becomes weaker.

4. *Glycogen.*

Glycogen in the smooth and striated muscle fibres in the lesion either disappears or decreases. Usually there is no change in the smooth and striated muscles of heart, liver and other parts.

5. *Endothelial cells of blood-vessels.*

Generally, there is no remarkable change. But there are pycnosis, caryorrhesis in the nuclei of endothelial cells of the focal lesion.

6. *Cells of reticulo-endothelial system.*

There is general swelling of reticulo-endothelium. Kupffer's cells of the liver are clearly visible. The reticular cells of spleen, bone marrow, and lymph nodes and the endothelial cells lining sinuses usually swell up and in some cases proliferates (increase of macrophages).

Distribution of Bacilli in the Cadaver.

These bacilli locate themselves mainly in lymphatic system, and are found in lymph space, pleural, peritoneal and other serous cavities.

CONCLUSION.

1. The change in the black-leg takes place mainly in muscles, and then in subcutis and skin. The tissue reaction is remarkably inflammatory at the last stage, and the serous transudation which is seen at the first stage becomes serofibrinous exsudation. Moreover it is accompanied by pseudoeosinophilic infiltration.

2. As the predisposed part of the lesion in the black-leg is the muscle, the most proper way of infection for the animal with gas-edema in intramuscular or subcutaneous injection.

3. The change in muscles may be roughly divided into three stages.

(1) Stage of cloudy swelling.

Cloudy swelling is produced in the muscle cells by exotoxin of bacilli. The interstitium is attended with haemorrhage and edema.

(2) Stage of waxy degeneration.

The muscle cells showing cloudy swelling are now mechanically broken into pieces and in fibrils by the movements of animals and the gas produced by bacilli.

The coagulative necrosis is the result.

(3) Stage of myolysis.

The muscle cells which are fallen into the coagulative necrosis are further liquefied, (colliquative necrosis) and a copious pseudoeosinophilic infiltration is produced.

4. Edema in this disease is mainly of toxic nature. Inflammatory edema also takes a part. Edema always precedes gas-formation.

5. "Ranziger Geruch" was not recognized in the black-leg cadavers.

6. The gas-formation is very slight and can be recognized only microscopically. No foamy organs were observed in various organs. The foamy organ formation is secondary in this disease. It is mainly post-mortal, or at least the agonial change and has no great significance.

7. The propagation of black-leg bacilli in the body is mainly through lymphatic channel, and the lesion is produced continually from the site of inoculation.

8. The bacilli could be proved in the circulating blood of the infected guinea-pig. But it is to be regarded as a case of passive transmission. Hence bacteriaemia in this disease has no significance.

9. On the contrary, the main change in the cortical and medullary layer of adrenals is different from the case of other toxinaemia, and in spite of inconspicuousness of the change of various organs, the change in this organ is of quite significant.

10. Toxins which can be imagined from the view point of morbid anatomy and histology are as follows: haemotoxin, leucocidin, toxin for endothelium of blood vessels and for cells of reticulo-endothelial system.

11. As *L. Aschoff* already advocated, it would be most proper that "Rauschbrand" is pathologico-anatomically placed under "gas-edema".

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氣腫疽病理ノ實驗的研究

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從來人ハ氣腫疽ニ不感ナリト信ゼラレタルモ這般ノ歐洲大戰ニ於ケルがすぶらんどノ研究ニヨレバ必ジシモ然ラザルガ如ク本菌並ニ類似菌ニヨル人ノ感染例少ナカラズ即がすぶらんどニ見ル病

理解剖的所見ハ必ズシモ一種ノ菌ニ限ルモノニアラズシテ種々ノ菌ニヨリテ構成シ得ルモノナリ L. Aschoff ハ人ノがすぶらんど馬ノ惡性水腫、牛ノ氣腫疽等ハ何レモ病理解剖的ニ同一所見ヲ呈スルモノニシテ病原學的ニ整理未濟ナル今日ニ於テハ總括シテがすぶらんどト稱スルヲ至當ナリト提唱セリ

余ハ嘗テ數多ノ氣腫疽可檢材料ヲ檢索シ所謂氣腫疽屍ノ呈スル病理解剖的所見ハ氣腫疽以外ノ菌ニヨリテモ生ジ得ルニ非ズヤト疑ヘリ此疑問解決ノ爲先ヅ氣腫疽ノ病理ヲ實驗的ニ海狸ヲ用ヒテ研究セリ其詳細ハ實驗醫學雜誌第8卷第10號ニ掲載セリ次ニ其結論ノミヲ抄録ス

1. 氣腫疽ニ於ケル組織ノ變化ハ筋肉ヲ最トシ皮下織及皮膚之ニ次グ組織反應ハ初期ハ中毒性ヲ帶ビ明瞭ナラザルモ末期ニ於テハ炎症反應著シ即初期ニ見ル漿液性滲漏ハ漿液纖維素性滲出トナリ加之擬「ゑ」白血球ノ浸潤ヲ伴フニ至ル

2. 氣腫疽ニ於テ病竈ノ好發部位ハ筋肉ナルヲ以テ動物ヲシテ氣腫疽ニ感染セシメントスル凡テノ實驗ニ於テハ接種部位ハ筋肉又ハ皮下ヲ以テ最モ適當トス

3. 筋肉變化ノ形成機轉ヲ約言スレバ先ヅ局所ニ増殖スル氣腫疽菌ノ產生毒素及該毒素作用ノ結果スル出血水腫等ニ因ル血行障礙ニヨリ筋纖維ノ抵抗減退ス(第1期)カ、ル筋纖維ハ伸展收縮等ノ單ナル器械的作用ニヨリ Schollige Degeneration ヲ生ズ此間隙内ニ侵入セル菌ハ發育ニ際シ瓦斯ヲ產生シ益々筋纖維破壊ヲ助長ス(第2期)此期ノ筋破壊作用ハ主トシテ器械的ニシテ菌ノ蛋白溶解酵素ハ之ニ關與セズ

Schollige Degeneration ニ於ケル筋斷片ハ先ヅ凝固壞死 Koagulationsnekrose ニ陥入り次デ更ニ融解性壞死 Kolliquationsnekrose ニ陥リ液化ス即 Myolyse ヲ生ズ而シテ菌ハ發育旺盛期ヲ過ギ漸

次退行型ヲ多ク認ムルニ至ルカ、ル組織ノ融解性壞死產物及死滅セル菌體成分ハ擬「ス」白血球ニ對シ陽性へもたしきす性ニ作用シ之ヲ索引シ該白血球ノ浸潤ヲ誘發ス(第3期)

筋肉液化ハ主トシテ壞死組織ノ自家融解酵素ニ因ルモノニシテ浸潤白血球ノ蛋白分解酵素モ亦之ニ參與ス菌ノ蛋白分解酵素ハ之ニ關與セズ

4. 水腫ハ主トシテ中毒性ニシテ炎性ヲ加味ス而シテ常ニ瓦斯產生ニ先立ツ

5. 氣腫疽屍ニ於テ Ranziger Geruch ヲ認メサリキ

6. 瓦斯產生ハ輕微ニシテ組織學的ニ始メテ認メラル筋肉ノ病竈ニ於テ既ニ然リ而シテ諸內臟ニ於テハ泡沫臟器ヲ認メタルコトナシ泡沫臟器形成ハ二次的ニシテ重大ナル意義ナシ主トシテ死後ノ變化ニ屬ス

7. 氣腫疽菌ノ體內ニ於ケル傳播系路ハ主トシテ淋巴道ニ依ルモノニシテ病竈ハ局所ヨリ連續的ニ生ズ血行ニヨル傳播ハ菌ノ靜脈內注射ノ例其他極メテ稀レニ認ムル所ニシテ實際上殆ンド顧慮ヲ要セズト信ズ

8. 氣腫疽感染海猿ノ血中ニ死前9時間ニシテ菌ヲ證明シタルコトアルモ其菌數極メテ僅少ニシテ培養的ニノミ陽性ナリキ斯ク菌數ノ極メテ小ナルコト及傳播系路ガ主トシテ淋巴道ニ依ルコト等ヨリ見テ流血中ノ菌ノ存在ハ本症ノ死因ニ關與スル所極メテ鮮シ從ツテ本症ニ於テハ菌血症ハ意義ナキモノト信ズ

9. 之ニ反シ副腎ノ皮質髓質ニ於ケル重要ナル變化ハ他ノ中毒症ノ場合ト異リ諸內臟ニ於テハ變化著明ナラザルニモ拘ハラズ毒血症ガ本症ニ於テハ可成意義アルヲ思ハシム

10. 病理解剖的及組織的所見ヨリ想起セラル、毒素ハ左ノ如シ
Haemotoxin, Leukocidin 及血管內皮細胞, 小血管壁竝ビニ網狀

織内皮細胞等ニ作用スル毒素等

11. Aschoff ハ嫌氣性創傷性感染病 Gasbrand, Gasphlegmone, Gasgangrän, Gangrène gazeuse, Gangrène foudroyante, Rauschbrand (Rind), Malignes Oedem (Pferd) 等ヲ Aetiologie ノ如何ニ拘ハラズ病理解剖學的ニ Gasoedem トシテ一括スルコトヲ提唱セリ即瓦斯產生ニ先立ち必ズ水腫ヲ生ジ主トシテ筋肉ノ變化ヲ伴フ創傷性感染病ヲ意味セリ余ハ氣腫疽モ亦病理解剖學的ニ Gasoedem ニ屬スルモノタルヲ確認セリ