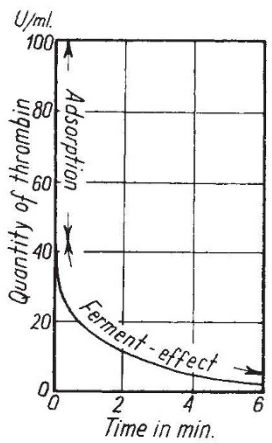

Fig. 1

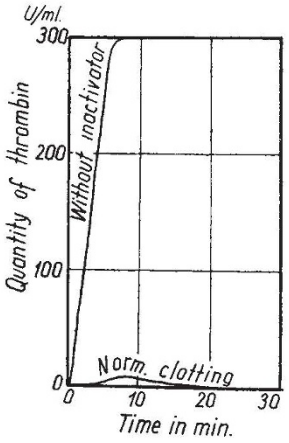

Fig. 2

In blood conserved for blood transfusions, the inactivator is almost completely lacking: thus it resembles thrombose-blood. This last observation agrees well with the data of Lenggenhager[4]. The inactivating capacity of thrombose-blood was shown in a 10-minute test. The durability of the thrombin preparations is in reverse proportion to the quantity of inactivator impurity.

Full details of this work will appear in the *Acta Physiologica Hungarica*.

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Inactivation of Antibacterial Substances with Cysteine

A WIDE range of therapeutic substances (for example, arsphenamine[1], 2-methyl-1 : 4-naphthaquinone[2], penicillin and other mould products[3,4] and tervalent antimonials[5]) is inactivated by cysteine. Colwell and McCall[2] found that cysteine antagonizes the antibacterial action of 2-methyl, 2-methyl-3-chloro- and 2 : 6-dimethyl-1 : 4-naphthaquinone on *B. coli*, but not that of 2-methyl-3-methoxy-1 : 4-naphthaquinone, and postulated that inactivation might be caused by reaction of the sulphydryl group from the cysteine at the 3-position of the quinone. Geiger and Conn[6] have suggested a somewhat similar mechanism for the inactivation of patulin and penicillic acid.

We considered that a polarographic investigation of the reaction with cysteine might provide additional information about the character of the reaction. Cysteine and cystine in an ammoniacal cobalt buffer solution yield a catalytic polarographic step, which disappears if the sulphydryl group enters into chemical combination[7]. By measuring the rate at which the height of the catalytic step decreases, the velocity of the reaction can be determined. The technique does not give information about the oxidation of cysteine to cystine, but this information can be obtained by combining the polarographic method with the sodium nitroprusside test.

The following technique was used to study the reaction between cysteine and a number of representative quinones and mould products: 1 ml. of 0·02 *M* cysteine hydrochloride was mixed with 1 ml. of a 0·02 *M* solution of the therapeutic substance and 4 ml. of a phosphate-citrate buffer solution (*p*H 7·0); the mixture was diluted to 20 ml. with air-free water and stored at room temperature in a rubber-capped bottle. A 1-ml. sample was withdrawn immediately by means of a graduated syringe and diluted to 10 ml. with air-free water. 1 ml. of the dilution was added to 10 ml. of a freshly prepared ammoniacal cobalt buffer solution (0·002 *M* cobalt chloride, 0·2 *M* ammonium hydroxide, 0·2 *M* ammonium chloride, and 0·02 per cent gelatin) and made up to 20 ml. with water, and a portion of it was examined in the polarograph. Further 1-ml. samples were withdrawn after 3 and 24 hours and tested in the same way. The experiment was repeated using an excess of the drug. The quinones were examined in 25 per cent aqueous dioxane solution. Each experiment was paralleled by a control experiment in which the drug was omitted.

When one gram-molecule of any of the drugs was used for every gram-molecule of cysteine, there was little effect on the catalytic step. Nevertheless a ten-fold excess of quinones, unsubstituted in the 3-position, and either substituted or unsubstituted in the 2-position (for example, benzoquinone, 1 : 4-naphthaquinone and 2-methyl-1 : 4-naphthaquinone), suppressed the step, indicating that a reaction had taken place. The reaction was not quantitative; but probably a portion of the cysteine had been oxidized to cystine, for the final solutions failed to give a mauve colour with sodium nitroprusside. Quinones substituted in both the 2- and the 3-positions (for example, phthiocol and phthiocol methyl ether) did not react with cysteine. Of the mould products tested, patulin (clavacin or claviformin) reacted more readily than crystalline sodium penicillin II (penicillin *G*); citrinin was least reactive.

It is thought that these preliminary results are of some interest, and that further development of the polarographic technique might provide important information about the mechanism of drug action.

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Chondrodystrophy (Achondroplasia) and Humoral Agents

IN *Nature*[1] and elsewhere[2,3], A. N. Studitsky has recently made statements to the effect that "studies of the interrelationship of the endocrine glands in the causation of experimental chondrodystrophy, taking into account the influence of the vitamin D balance, prove almost certainly the hypothesis of the humoral nature of this disease". More specifically it may, according to Studitsky, be concluded "that the achondroplasia of the chick embryo is a hyperparathyroid dystrophy of the skeleton, developing against the background of avitaminosis D", and the author continues that "it does not seem improbable that our hypothesis as to the nature of fœtal achondroplasia can also be extended to the analogous affection in man". It is the purpose of this note to point out very briefly that one of the few conclusions which available evidence permits one to draw concerning the causation of chondrodystrophy is that humoral agencies play no part in the origin of this skeletal abnormality.

The statements of Studitsky are based on two lines of evidence, namely, observations on chorio-allantoic transplants of bones from heterozygous Creeper fowl embryos and skeletal reactions of normal chicken embryos bearing chorio-allantoic pituitary transplants.

I have shown[4] that the skeletal peculiarities of Creeper fowl consist in a chondrodystrophy-like condition. Studitsky reports that tibiæ of 7½- and 8-day Creeper embryos transplanted to the chorio-allantois of normal hosts take an entirely normal course in regard to cartilage differentiation, bone formation, and other features. He arrives at the conclusion[2] that: "Les difformités du squelette creeper ne sont pas encore déterminée aux stades précoces du développement. Elles apparaissent à la suite d'un retard du développement des tissus osseux et cartilagineux, provenant sans doute des conditions humorales qui se sont formées dans l'embryon creeper en croissance".

There is little reason to doubt that the heterozygous and homozygous expressions of the Creeper mutation reflect quantitatively differing effects of the *same* fundamental aberration from normal development. Since homozygous Creeper embryos generally die at the end of the third day of incubation, that is, prior to the appearance of any glandular tissues, endocrine factors are excluded as a possible primary cause. It is for this reason that a study of the endocrine glands of Creeper fowl seemed pointless. Moreover, there is ample experimental evidence for the conclusion that the homozygous as well as the heterozygous features of Creeper fowl extremities are fully determined long before endocrine factors can play any part, and for the further conclusion that these features remain uninfluenced by the humoral environment of normal hosts. Experiments by Hamburger[5], with a technique incomparably superior to that of chorio-allantoic transplantation, have shown that the heterozygous and homozygous peculiarities of Creeper limbs are fixed "at least as early as the fifty-fourth hour of incubation and cannot be reversed by implantation into a genetically normal host". Rudnick[6] arrived at essentially the same conclusion in grafts from 6–19 somite stages of heterozygous and homozygous Creeper embryos, that is, stages prior to the establishment of embryonic circulation. It can, therefore, be stated with assurance that humoral factors have no part in determining the chondrodystrophic features of the extremities of Creeper fowl.

In an attempt to analyse the origin of sporadic (that is, non-hereditary) chondrodystrophy of developing chicks Studitsky transplanted fragments of pituitary gland on to the chorio-allantoic membrane of normal seven- and eight-day embryos. He reports that he obtained by this method a significant excess in incidence of sporadic chondrodystrophy, as compared with unoperated controls, and he interprets his observations as due to pituitary stimulation of the host's parathyroid glands, in the presence of a vitamin D deficiency and a resulting interference with normal skeletal development.

It cannot be determined from the description of Studitsky's material whether or not the abnormal embryos which he found show the histological criteria of chondrodystrophy. The fact that he makes reference in this connexion to a micromelia of nutritional origin, described by me[7], without mentioning that this micromelia (afterwards found to be due to manganese deficiency) was reported as being 'quite distinct' from sporadic chondrodystrophy, does not inspire confidence. It should be noted also that Pighini[8], after even earlier pituitary transplants, did not obtain results comparable to those of Studitsky. However, even if it were granted that the experimental conditions to which Studitsky subjected his embryos led to an appreciable incidence of micromelia, and if it were granted further that this micromelia bears resemblance to chondrodystrophy, for which there is no