positive diazo reaction and the occurrence of organically bound sulphur which could be oxidized by bromine to inorganic sulphate. This suggested to us that the reducing substance may be the imidazole base ergothioneine (the betaine of thiolhistidine), first discovered by Tanret[1] in ergot, and later shown to occur in low concentrations in red blood corpuscles[2–4]. The application of certain analytical procedures for the determination of ergothioneine[5–7], both in the vesicular secretion and blood of boars, showed that the level of ergothioneine in the vesicular secretion averages 40 mgm. per cent as against 6 mgm. per cent in the blood.

The vesicular secretion collected from the seminal vesicles of four boars (1,300 ml.) was diluted with an equal volume of water and centrifuged; the supernatant was deproteinized with zinc hydroxide[8], the extract concentrated to 400 ml., precipitated with an equal volume of ethanol, the alcoholic filtrate concentrated to 200 ml., treated with 10 ml. 10 *N*-sulphuric acid, filtered again, and the water-clear and colourless solution precipitated with a 50 per cent solution of phosphotungstic acid. The precipitate was decomposed with barium hydroxide, and the solution, after removal of barium, was concentrated *in vacuo* to a syrupy consistency. On gradual addition of ethanol and cooling, crystallization set in, and after two days at 5° C., 480 mgm. of crystalline material was obtained. This gave two further reactions characteristic for ergothioneine, namely, a crystalline derivative with mercuric chloride, and trimethylamine on treatment with alkali. After two recrystallizations from 66 per cent ethanol and drying at 105° C., the substance was analysed—found: C, 47·14; H, 6·57; N, 18·24; S, 14·02. $C_9H_{15}N_3O_2S$ requires: C, 47·14; H, 6·59; N, 18·33; S, 13·98 per cent.

The correlation between the reducing power and ergothioneine content in the seminal fluid of other animals is being examined; but preliminary experiments indicate considerable variations in this respect between species.

This investigation was carried out on behalf of the Agricultural Research Council; one of us (E. L.) is indebted to the British Council for a scholarship, and to Prof. D. Keilin for hospitality in this Institute.

E. Leone
T. Mann

Molteno Institute,
Cambridge. May 29.

[1] Tanret, C., *J. Pharm. Chim., Paris*, **30**, 145 (1909).
[2] Hunter, G., and Eagles, B. A., *J. Biol. Chem.*, **65**, 623 (1925).
[3] Eagles, B. A., and Johnson, T. B., *J. Amer. Chem. Soc.*, **49**, 575 (1927).
[4] Benedict, S. R., Newton, E. B., and Behre, J. A., *J. Biol. Chem.*, **67**, 267 (1926).
[5] Brown, H., *J. Biol. Chem.*, **158**, 601 (1941).
[6] Hunter, G., *Biochem. J.*, **22**, 4 (1928); **48**, 265 (1951).
[7] Touster, O., *J. Biol. Chem.*, **188**, 371 (1951).
[8] Mann, T., *Biochem. J.*, **40**, 481 (1946).

Inactivation of Adrenotropic Hormone (ACTH) by Plasma

The rapid disappearance from the blood of administered adrenotropic hormone (ACTH) has been demonstrated by Sayers *et al.*[1] using an infusion technique on human subjects, and Greenspan *et al.*[2], after intravenous injection into rats.

In view of the importance of these findings, we would like to report that we have observed the rapid inactivation of an adrenotropic hormone preparation during incubation with heparinized plasma from rats, rabbits and humans. Parallel incubation of the same preparation with the solvent containing an equivalent amount of heparin showed no measurable inactivation when assayed by the Sayers adrenal ascorbic acid depletion assay[3].

The Effect of Incubation at 37° C. for 5 min. on an ACTH Preparation with 80 per cent Plasma from Various Sources

Percentage of inactivation		
Rat	Rabbit	Human
74·9	93·5	90·6

These results will have to be borne in mind when attempting to assay the adrenotropic hormone content of blood of these species.

Max Reiss
F. E. Badrick
I. D. K. Halkerston
C. Plaice

Biochemical and Endocrinological
Research Department of
Bristol Mental Hospitals,
Fishponds, Bristol.
April 7.

[1] Sayers, G., Burns, T. W., Taylor, F. H., Jager, B. V., Schwartz, T. B., Smith, E. L., Samuels, L. T., and Davenport, H. W., *J. Clin. End.*, **9**, 593 (1949).
[2] Greenspan, F. S., Choh Hao Li, and Evans, Herbert M., *Endocrinol.*, **46**, 261 (1950).
[3] Sayers, G., Sayers, M. A., and Woodbury, L. H., *Endocrinol.*, **42**, 379 (1948).

Sensitivity to Tuberculin

While a reasonable account can now be given of most stages of the mechanism of the urticarial type of skin response to injected antigens, the observed facts concerning the tuberculin or delayed type of reaction cannot yet be adequately explained. One of the main difficulties has been the failure to demonstrate circulating antibodies by the classical Prausnitz–Kuestner technique. However, Chase[1] showed that sensitivity to tuberculin could be passively transferred by the intra-peritoneal or intravenous injection of white cell concentrates from sensitized animals. More recently, this finding has been confirmed by Lawrence[2], who achieved passive transfer in the human by the intradermal injection of white cells from sensitized donors.

On the basis of tissue culture experiments certain workers, notably Rich and Lewis[3], Moen and Swift[4], had previously suggested a theory of 'tissue' or 'cellular' sensitivity to tuberculin, having demonstrated *in vitro* a cytotoxic action of tuberculin upon tissues from animals with experimentally induced tuberculosis. They found unequivocal evidence of the toxic action of tuberculin upon 'sensitized' wandering cells and observed that, while fibroblasts grew out in culture, their growth-rate was reduced and their cytological appearances altered. Aronson[5] and Raffel[6] demonstrated that bone marrow explants from sensitized animals were killed by tuberculin *in vitro*. No susceptibility of cellular elements to the antigen has been demonstrated *in vitro* when the urticarial or Arthus type of sensitivity has been investigated by tissue culture methods.

The development of a technique for growing explants of adult skin in a fluid medium by Medawar[7] has provided an opportunity for observing the effect of tuberculin upon skin *in vitro*. This technique permits a closer analogy to be drawn between the events following intradermal injection of tuberculin *in vivo*