women should be told frankly that if they smoke they not only put their own lives in jeopardy but, if they continue to do so during pregnancy, also expose their unborn infants to an unnecessary risk."

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COMPLICATIONS OF DEPRESSED SKULL FRACTURE

SIR,—May I comment on one group of patients mentioned in the interesting paper by Mr. Miller and Professor Jennett (Nov. 9, p. 991)? In 3 cases fractures were not elevated because the wounds had already been sutured in the casualty department. I agree that this policy of surgical inactivity is in line with traditional teaching but am fairly sure that it is high time for a change. All 3 of the Glasgow patients developed severe intracranial infection and 1 died. Although I have not analysed my own cases I can recall several in whom non-intervention once wounds have been closed has been followed by osteomyelitis, abscess formation, and meningitis. The risk nowadays of spreading infection by operating is surely far less than the risk run by accepting an unsatisfactory and dangerous situation. Naturally, one would like to think that the day may come when all compound skull fractures will be recognised and treated adequately within a few hours of wounding, but until that happy time arrives I suggest that operation should be performed on these fractures whether the skin has been sutured or not.

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ORAL CONTRACEPTIVES AND CÆRULOPLASMIN ACTIVITY

SIR,—During a survey of the quantitative levels of cæruloplasmin activity (assayed, using paraphenylene diamine as a substrate, in a 'DU-2' Beckman spectrophotometer) in the sera of individuals belonging to various races, very high values for cæruloplasmin-oxidase activity were found in women regularly receiving oral contraceptives. A comprehensive study was conducted with three control groups: (*a*) women of comparable age and race, married, and using other forms of contraception, such as diaphragm, intrauterine device, and safe-period method; (*b*) female members of the same families as the index women; (*c*) three women whose levels were ascertained during and after use of oral contraceptives.

The serum-cæruloplasmin levels, in mg. per 100 ml. ±S.D., was 72·75±1·7 in 12 women receiving oral contraceptives, and 34·68±3·9 in 15 women using other contraceptive methods. The female members of comparable age in the families of the index women had normal levels of serum-cæruloplasmin, thus excluding the possibility of the high levels of the latter being hereditary. The serum-cæruloplasmin values in three women during and after use of oral contraceptives were as follows:

Case no.	*While taking oral contraceptives*	*After taking oral contraceptives*
1	72·24	36·12
2	68·8	31·82
3	71·8	34·2

We have studied the immunochemical implications of the raised cæruloplasmin level. We found that the specific activity of the protein, calculated as the ratio of mg. per 100 ml. of activity to immunochemical units of protein (estimated in immunodiffusion double-agar diffusion tubes using specific antihuman cæruloplasmin antiserum), is equal to that of normal-level cæruloplasmin. This is evidence for increased level of the protein rather than enhanced activity.

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SERUM-ALBUMIN AND INSULIN ANTAGONISM

SIR,—Patients with essential diabetes mellitus have increased insulin antagonism associated with their plasma-albumin—an exaggeration of that also found in the albumin fraction of healthy people. In concentrations of 3·5–5·5 g. per 100 ml., both diabetic and normal albumin completely inhibit the effect of 1000 microunits insulin per ml. added to the incubation medium of the isolated rat diaphragm. At 1·25 g. per 100 ml., however, the albumin from essential diabetics (including pre-diabetics) is still highly antagonistic, whereas that prepared from healthy people is inactive.[1,2] Also tested in this way, the albumin from normal consanguineous relatives of diabetic patients has shown insulin antagonism.[3] The albumin in the above studies was usually prepared by the trichloroacetic-acid/ethanol method of Debro et al.,[4] which is a lengthy procedure involving dialysis for 72 hours with subsequent lyophilisation; moreover the end-product is not completely soluble.

In 1966 Fernandez et al.[5] showed that a mixture of ethanol and hydrochloric acid gave an essentially complete precipitation of globulin from a solution of globulin and albumin. After removal of the precipitated globulin by centrifugation, the albumin was recovered from the supernatant by precipitation with sodium acetate prepared as an ethanol solution. We have adopted this principle for the preparation of albumin from the serum of normal subjects and diabetic patients. The end-product is a finely divided off-white powder, easily soluble in water and physiological buffers, and only one protein band is obtained after electrophoresis in barbiturate buffer pH 8·6 and subsequent staining with nigrosin.

Serum.—Venous blood was withdrawn into a dry syringe from fasting donors. After clotting had occurred, all samples were centrifuged for 15 minutes at 1400 *g*, to separate the serum.

Extraction.—5 ml. volumes of serum were ordinarily used, and whenever stirring or thorough mixing was required this was effected by a small polytetrafluoroethylene-covered magnet. The globulin fraction was precipitated by adding the serum drop by drop to a flask containing an agitated volume of hydrochloric-acid/ethanol reagent (1 ml. concentrated hydrochloric acid in 600 ml. ethanol) equal to 9 times the volume of serum. The flask was closed with 'Parafilm' and incubated in a water-bath at 37°C for 30 minutes. The globulins were then removed by centrifugation for 15 minutes at 2600 *g*. Precipitation of the albumin from the isolated supernatant was effected by the addition, with stirring, of 0·1 *M* sodium-acetate/ethanol reagent, the volume used being a fifth of the volume of the supernatant. Centrifugation at 1400 *g* for 5 minutes was sufficient to collect the precipitate, which was then washed with 20 ml. of methanol, followed by 20 ml. of a mixture of methanol and diethyl ether 3/1 v/v. After collection by centrifugation the albumin was given a final wash with 20 ml. diethyl ether to remove the methanol. The albumin was resuspended in 10 ml. diethyl ether and collected by vacuum filtration on a sintered glass disc. When the bulk of the ether had been removed and the layer of albumin was starting to crack, the vacuum was broken and the preparation gently ground to a

ALBUMIN ASSAYS OF THE DIFFERENT GROUPS

Origin and status of albumin tested at 1·25% (no.)	Mean glucose uptake above basal level * (±S.E.M.) (mg. glucose per 100 ml. per 10 mg. rat diaphragm)	
	Buffer+1000 microunits per ml. insulin	Albumin in buffer +1000 microunits per ml. insulin
Group I:		
Antagonistic (27) ..	13·14 (±0·29)	6·23 (±0·48)
Non-antagonistic (3)	12·06 (±0·87)	10·07 (±0·56)
Group II:		
Antagonistic (14) ..	12·15 (±0·71)	5·46 (±0·62)
Group III:		
Antagonistic (4) ..	13·80 (±0·98)	8·07 (±1·26)
Non-antagonistic (18)	12·05 (±0·41)	12·26 (±0·47)

* Amount of glucose taken up by diaphragm when no insulin is present in incubation medium.

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