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EFFECTS OF INSULIN, GLUCAGON, AND INSULIN/GLUCAGON INFUSIONS ON LIVER MORPHOLOGY AND CELL DIVISION AFTER COMPLETE PORTACAVAL SHUNT IN DOGS

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Summary Insulin, glucagon, and insulin/glucagon mixtures have been infused for four days into the left portal vein of dogs after portacaval shunt. In the left but not the right liver lobes, insulin alone reduced atrophy, preserved hepatocyte ultrastructure, and trebled cell renewal. Glucagon alone had no effect. In small doses, glucagon did not potentiate the action of insulin and in large doses it may have reduced the insulin benefit. These studies explain the development of the previously mysterious Eck fistula syndrome, provide clues about in-vivo cell growth control by hormones, and suggest new lines of inquiry about the pathogenesis and/or treatment of several human disease processes.

Introduction

A COMPLETELY diverting portacaval shunt (Eck's fistula) profoundly alters the structure and function of the liver in several species.[1] Until recently, these changes were thought[2-6] in what has been termed the "flow hypothesis" to be caused by and to be proportionate to the reduction in total hepatic blood-flow which the procedure causes.[4] We have suggested instead that disruption of normal endogenous insulin delivery to the liver was mainly responsible[7-9] and in a preliminary communication we showed that infusion of commercial insulin into the tied-off central portal vein of dogs prevented most of the acute atrophic changes that are ordinarily very advanced by light microscopy within four days after portacaval shunt.[10]

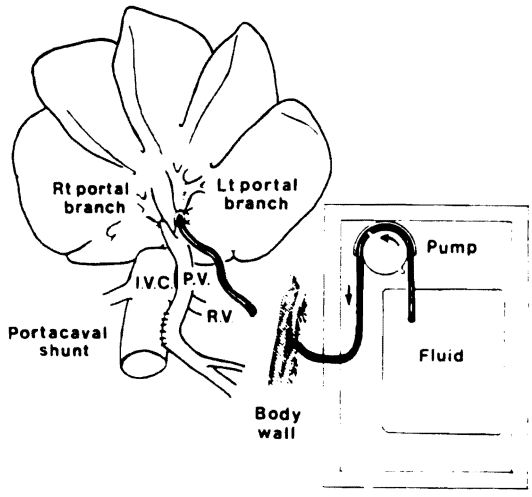
In this investigation, more complete studies were performed in dogs of hormone infusions after Eck's fistula. Insulin, glucagon, and combinations of these two agents in various doses were tested for their effect upon the morphology and division-rates of the hepatocytes.

Methods

Anæsthesia for operation and later killing was with sodium pentobarbitone supplemented with phencyclidine hydrochloride ('Sernylan') and succinylcholine chloride ('Anectine'). Large side-to-side portacaval shunts were constructed with excision of ellipses from both the portal vein and the inferior vena cava. The shunts were made completely diverting by individually ligating the main right and left portal trunks. Frequent morning blood-sugars were determined during the period of study by means of an 'AutoAnalyzer'.

The experimental groups of mongrel dogs are shown in table 1. In groups 2–9, the tip of a fine infusion catheter was placed into the tied-off left portal branch and led through the body wall and through a long subcutaneous tunnel to a small calibrated finger pump that was incorporated into a light body cast (see accompanying figure). The infusion volumes never exceeded 25 ml per day. Regular commercial insulin from beef and pork pancreas was used for much of the work. To rule out artefacts from contaminants, research-quality bovine insulin prepared by the Eli Lilly Company (Indianapolis) was used in group 5 (lot no. 24C–3130). The glucagon which was pure (Lilly lot no. 95C–0314) came from beef and pork pancreas. The small and large glucagon infusion doses in the glucagon-alone groups 6 and 7 respectively would have provided approximately a 2/1 or 2/100 insulin/glucagon molar ratio had insulin been given in the amounts used in groups 3 and 5. A 2/1 ratio is in the physiological range.[11]

Biopsy specimens were obtained from a left lobe at the time of portacaval shunt (LO), and from left and right lobes (L4 and R4) at the time of killing. About two hours before killing, 63 of the 70 dogs were given 4·5 mC of (CH_3-^3H) thymidine by the intravenous route. The specific activity of the thymidine was 6·4 C per mmol. Because the same dose was always used without regard to animal size, the dose spectrum per kilo-



Experimental arrangement in groups 2–9.

I.V.C.=inferior vena cava. P.V.=portal vein. R.V.=renal vein.

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gramme of body-weight was broad, ranging from 0·16 to 0·48 mC. Shunt patency and catheter position were always verified at necropsy.

After fixation in formalin, frozen sections were cut from the samples of liver tissue and stained with sudan-4 for fat. The remaining tissue was then processed and the paraffin sections were stained by a variety of methods which have been described previously.[7,9] Autoradiography was carried out on paraffin sections using Kodak NTB2 liquid emulsion and an exposure-time of 28–35 days. Small cubes of each hepatic sample were used for electron microscopy. Initial fixation was glutaraldehyde followed by osmic acid. After embedding in 'Epon', ultrathin sections were cut, stained with lead citrate, and exmined in a Philips 300 electron microscope. The size of the hepatocytes was determined by tracing out large numbers of midzonal liver cells on standard-thickness paper, cutting out the silhouettes, and weighing them. The weights were referred to as size units. We have shown this to be an accurate method for comparing cell sizes by confirmatory planimetry and by studies of unicellular organisms, the size of which could be directly determined. Midzonal hepatocytes were also used for measuring on electron micrographs the length of rough endoplasmic reticulum per area of cytoplasm by Loud's method.[12]

Results

Controls and Placebo Infusions

Four days after portacaval shunt, the liver cell size units in both the left and right lobes of the animals of group 1 decreased by about half (table I). In addition to the hepatocyte shrinkage, light-microscopic examination showed that the hepatocytes were irregular in shape and were depleted of glycogen. The lobules were smaller than normal and the reticulin framework was condensed around the central veins. The Kupffer cells were enlarged and filled with material which included ceroid and lipofuscin and in three animals hæmosiderin. A little fat was visible in the hepatocytes in 5 of the 11 dogs. The number of hepatocyte mitoses was greater than normal.

The ultrastructural changes caused by Eck fistula within the four days were the same in the left and right lobes. The most prominent effects were decreases in the amount of rough endoplasmic reticulum with disruption and dilatation of the cisternæ. Morphometric analysis showed that the area of rough endoplasmic reticulum per volume of cytoplasm was reduced to between half and a third the quantity found in the preoperative biopsy and the number of membrane-bound ribosomes was reduced. The quantity of smooth endoplasmic reticulum was increased. Some of the mitochondria were enlarged and showed distortion of their cristæ.

The autoradiographic studies gave quantitative confirmation of the impression of an increased mitosis-rate (table II). The number of hepatocytes undergoing D.N.A. synthesis in both left and right liver lobes was nearly 5 per 1000 (table II). The rate in 17 normal dogs including 6 that were subjected to sham dissections of the portal tract was recently established in our laboratories as 1·6±0·4 (S.D.) per 1000 cells in the left lobes and 1·6±0·5 (S.D.) in the right lobes.[9]

All of the foregoing findings were almost precisely the same in the dogs of group 2 whose left lobes were infused with a heparin saline infusion for four days after Eck fistula (table I and II).

TABLE II—CELL DIVISION BY AUTORADIOGRAPHY IN THE LIVERS OF 63 DOGS

Group no	No. of experiments	No. of labelled hepatocytes per 1000 hepatocytes mean±S.D.†		P values* L4 vs R4
		Left†	Right†	
1	8	4·9±1·0	4·7±0·9	N.S.
2	6	4·6±0·8	4·7±0·9	N.S.
3	9	13·0±3·9	4·6±0·9	<0·001
4	4	15·6±2·0	5·3±1·0	<0·001
5	8	14·4±1·1	4·8±1·0	<0·001
6	8	4·9±0·9	4·3±0·6	<0·05
7	6	4·2±1·5	4·3±1·1	N.S.
8	7	11·8±1·2	4·5±0·8	<0·001
9	7	14·8±1·0	4·5±0·9	<0·001

*Comparisons were by Student's *t* test.
†In 17 unaltered or sham-operated dogs the left and right lobar values respectively were 1·6±0·4 and 1·6±0·5. The increase caused by Eck fistula is significant (P<0·001).

Insulin Infusion

The infusion of 0·43 units per kg/day of commercial insulin into the left portal vein for four days after Eck fistula in group-3 dogs markedly reduced the hepatocyte shrinkage in the left lobes without affecting at all the acute atrophy of the right lobar hepatocytes. The amount of glycogen in the cytoplasm of the hepatocytes in the left lobe was still less than normal, but much more than in the atrophic right lobar hepatocytes. The Kupffer cells in both lobes were enlarged and full of ceroid and lipofuscin.

Ultrastructurally, comparisons of the left and right lobar hepatocytes showed that the liver cells exposed to insulin infusion were almost normal. The area of rough endoplasmic reticulum per volume of cytoplasm and the number of membrane-bound ribosomes were only slightly reduced; dilatation and disruption of the cisternæ were minimal. Glycogen granules were frequent, but the number of small fat vacuoles was increased. The

TABLE I—EXPERIMENTAL GROUPS AND HORMONE DOSES

Group no	No. of experiments	Dog weights kg mean±S.D.	Type of portal infusion*	Insulin dose units/kg day mean±S.D.	Glucagon dose mg/kg day mean±S.D.	Cell size units mean±S.D.			P values† L4 vs R4
						LO	L4	R4	
1	11	18·0±5·3	No treatment	0	0	0·197±0·04	0·108±0·02	0·108±0·02	N.S.
2	6	18·3±5·1	Heparinised saline	0	0	0·200±0·04	0·104±0·02	0·104±0·01	N.S.
3	13	17·8±3·4	Large dose insulin	0·43±0·05	0	0·189±0·03	0·160±0·02	0·100±0·02	<0·001
4	4	17·5±3·5	Small dose insulin	0·16±0·11	0	0·194±0·04	0·143±0·02	0·094±0·01	<0·05
5	8	17·0±2·9	Purified insulin	0·42±0·08	0	0·196±0·04	0·158±0·01	0·095±0·02	<0·001
6	8	18·9±2·9	Small dose glucagon	0	0·0053±0·0011	0·189±0·01	0·103±0·01	0·103±0·01	N.S.
7	6	17·5±2·7	Large dose glucagon	0	0·60 ±0·10	0·165±0·03	0·085±0·01	0·082±0·01	N.S.
8	7	15·0±2·4	2·1 insulin/glucagon	0·45±0·03	0·0053±0·0005	0·211±0·05	0·156±0·05	0·094±0·02	<0·05
9	7	17·6±4·1	2/100 insulin/glucagon	0·42±0·01	0·50 ±0·02	0·215±0·04	0·114±0·03	0·073±0·02	<0·05

*All insulin was commerical regular insulin, except in group 5. †Comparisons were by Student's *t* test.

TABLE III—MORNING BLOOD-SUGARS DURING FOUR-DAY EXPERIMENTAL PERIOD

Group no.	No. of determinations	Blood-sugar (mg/dl): mean ± S.D.
Normal dogs 1 and 2	10	61 ± 9
	20	63 ± 8
3	38	65 ± 13
4	13	71 ± 8
5	21	71 ± 11
6	24	65 ± 12
7	12	65 ± 10
8	24	63 ± 9
9	24	68 ± 13

changes in the hepatocytes in the right lobe did not differ from those seen in the controls.

Autoradiography showed that in the insulin-infused left lobes the number of cells undergoing D.N.A. synthesis had nearly trebled whereas D.N.A.-synthesising cells in the right lobes of the group-3 dogs were not different in numbers from those in the untreated Eck fistula animals of groups 1 and 2.

The results in all the end-points measured were almost exactly the same in the dogs of group 5 when purified insulin was used instead of the commercial insulin (tables I and II). A pronounced but slightly reduced protective effect of the left lobes was still evident even when the insulin dose was reduced in group 4 to about a third of that used in groups 3 and 5 (tables I and II).

In all the groups morning blood-sugars on frequent occasions did not deviate significantly from the normals in our laboratory (table III).

Glucagon Infusion

The administration of small (group 6) and large (group 7) doses of glucagon into the left portal vein for four days did not appreciably influence any of the changes caused by Eck fistula (tables I and II).

Insulin/Glucagon Infusion

When glucagon was added to proven effective doses of insulin to make an insulin/glucagon molar ratio of 2/1 (group 8), the effect was no different than with insulin alone (tables I and II).

The animals of group 9 received 100 times as much glucagon. Now the protection from hepatocyte atrophy in the left lobes afforded by insulin seemed partly lost. By one-way analysis of variance using Duncan's multiple-range test with 95% confidence limits, the L4 hepatocytes were not in the same subset as in the protected groups 3, 5, and 8, but neither did they fit with the control and pure glucagon groups 1, 2, 6, and 7. The badly affected left lobar liver cells were still significantly less shrunken than those on the right (table I). The autoradiographic observations with D.N.A. incorporation showed the same threefold increase in the left lobes as with insulin alone or as with the 2/1 insulin/glucagon ratio (table II).

The light and electron-microscopic analysis did not show why the insulin protection had been partly lost in group 9. The amount of glycogen in the left lobar hepatocytes seemed almost normal, but the volume of rough endoplasmic reticulum was less than in the hepatocytes in the left lobes of dogs infused with insulin alone or insulin combined with lower doses of glucagon. Some mitochondrial abnormalities were also present.

Discussion

Eck thought[13] that a completely diverting portacaval shunt (Eck's fistula) in dogs was compatible with prolonged good health. This conclusion was refuted in 1893 by Hahn, Massen, Rencki, and Pawlow[14] whose dogs with Eck fistula developed anorexia, weight loss, liver atrophy, and hepatic encephalopathy. The inability to explain these consequences caused Bollman[15] to write in 1961: "In the 83 years since it was first reported, the Eck fistula has been reasonably successful in hiding its secrets as well as in giving rise to many additional questions fundamental to an understanding of the functions of the intestine, liver, and brain." By then it had been widely accepted that the Eck fistula syndrome was caused by a suboptimal volume as opposed to quality of hepatic blood-flow. This conclusion had apparent support from the fact that the portoprival complications could be minimised if the central tied off portal vein was revascularised with systemic venous blood (Child's portacaval transposition[3]) or with arterial blood.

The flow concept was undermined by a series of investigations in which two canine livers or fragments of the same liver were given different kinds of portal inflow.[7-9 16-20] The hepatocytes perfused with total portal flow or its pancreaticogastroduodenosplenic fraction became glycogen-rich, hypertrophic, and more active in cell renewal than the hepatocytes in the disadvantaged liver tissue which developed the histopathological features caused by Eck fistula despite apparently adequate replacement flow. Collectively, the enriching constituents thus demonstrated to be in portal blood were called hepatotrophic substances. It became obvious from biochemical[7 20] and pancreatectomy or alloxan-diabetes studies[8 9 20] that insulin was the most important of these substances and that the well-known efficiency of insulin's removal during a first pass through hepatic tissue[21-23] made it relatively unavailable for a second liver or liver fragment. At the same time the benefit after portal diversion from flow augmentation procedures such as Child's portacaval transposition was explained. If insulin and other hepatotrophic substances were bypassed around a single liver, they would be returned to it in diluted form in direct relation to the total hepatic blood-flow.

The natural deduction from our earlier studies was that many of the secrets of the Eck fistula were manifestations of an altered hormone environment in which the most important change was deprivation of the liver of direct access to endogenous insulin. The experiments of a recent preliminary communication[10] and of this more complete report directly tested that hypothesis and with unequivocal results.

Non-hypoglycæmic insulin infusions for four days into the tied-off left portal vein after Eck fistula greatly reduced the left lobar hepatocyte atrophy, permitted the ultrastructure of the protected liver cells to remain essentially normal, and caused a threefold increase in the number of left lobar hepatocytes undergoing mitosis. There was no spillover effect in the right lobes. Glucagon by itself did not cause any of these changes, and it did not potentiate them when added to insulin at a 2/1 molar insulin/glucagon ratio. At a dose 100 times greater, glucagon may actually have reduced the benefit from insulin.

Thus, it has been established that "hepatic insulino-

penia" is the most important element in the liver injury of Eck fistula. It is ironic that this answer was so close to detection all the time. Hahn et al.[14] definitely emphasised more than 80 years ago that, unless the portal vein was ligated above the last portal branch during the performance of Eck fistula, their dogs remained quite normal. This highest vessel drains the pancreas, but of course the endocrinological significance of that fact was not then known. Because of that same ignorance of hormones, speculation by Rous and Larimore[24] was vague about the possibility as they saw it in 1920 that portal blood might have special liver-supporting constituents. A perplexing observation of our own from almost 20 years ago contained a strong clue.[25] It was noticed that dogs with Eck fistula plus insulin-treated alloxan diabetes remained in much better health than animals with Eck fistula alone in that the diabetic dogs gained weight and were spared all evidence of encephalopathy. The now-obvious explanation is that peripheral insulin concentrations of two to three times normal are required in diabetics to maintain normoglycæmia when insulin is given systemically,[26] thus inadvertently providing a compensatory hepatic arterial increase in the hormone. Conversely, in untreated alloxan-diabetic rats with unaltered hepatic circulation, Reaven, Peterson, and Reaven[27] have demonstrated acute ultrastructural changes in the liver cells that were remarkably like those caused by Eck fistula.

In the past, we have emphasised[7-10 20] that multiple factors including collaborating hormones as well as nutrients undoubtedly contribute to the total hepatotrophic effects of portal blood. It would be regrettable now if the very clarity with which insulin has emerged as a principal portal hepatotrophic substance were to obscure the search for contributory factors. The observation that the insulin protection in our experiments was not quite complete may be a reflection of missing ancillary substances although this could also be explained by the inability of infusion pumps to make appropriate physiological modulations of delivery.

However, it is particularly in the area of cell growth control and regeneration that efforts must not be made to use insulin as a monolithic explanation for everything.[9] This can be made clear with some observations about hepatocyte renewal under differing portoprival conditions. In livers of Eck fistula dogs of the present study, in rat livers after portacaval shunt,[28] and in the portoprival hepatic fragments of double liver models[8 9] the atrophic and insulin-starved hepatocytes have been demonstrated by autoradiography to have mitosis-rates that paradoxically were three or four times normal. The stimulus for the low-grade hyperplasia is unknown, but it is presumably a response to an increased hepatocyte death-rate. The provision of exogenous insulin in the present studies or of endogenous insulin in the complicated double liver fragment experiments[8 9] provoked a sustained burst of proliferation beyond the already heightened mitotic background.

These results were consistent with those in the important study by Younger, King, and Steiner[29] who allowed rats to be alloxan-diabetic for a month before treating them with insulin. Although the livers were thought to already contain a higher than normal number of hepatocytes, the proliferative response to insulin was spectacular, being similar to that after a 68% liver resection. However, Younger and his colleagues[29] also showed that diabetic rats retained a potent although subnormal ability to regenerate their livers after an actual hepatic resection even if insulin treatment was withheld. Accordingly, it is unlikely that any single control factor will be the sole explanation of regeneration. Holley[30] and Leffert[31] have summarised the dozens of substances, hormonal (including insulin) and others, that can initiate and regulate cell growth in tissue-culture systems. There is no reason to doubt the relevance of their comments concerning the complexities of growth control to in-vivo situations.

Even so, the prospect seems promising of favourably influencing regeneration and recovery after acute liver injury in laboratory animals and ultimately in man by the simple expedient of intraportal insulin therapy. Earlier[8 10] we speculated that insulin for this purpose might have an augmented benefit if combined with glucagon. A recent study on regeneration by Bucher and Swaffield[32] has provided support for such combination therapy. However, the failure in our studies reported herein, as well as in past equally well-controlled ones,[8 9 20] to identify a beneficial additive role of glucagon, coupled with the slight possibility that in high doses it might have even cancelled some of the insulin benefit, would cause us either to omit glucagon or to use it in small amounts only.

As such possibilities are considered, the relevance of canine portal physiology to man will be raised. It has been pointed out elsewhere[1] that the same general light and electron microscopic changes have now been seen after portal diversion in the livers of rats, dogs, baboons, and humans with some variations in degree. Thus, the hepatic injury of Eck fistula is common to all species so far studied. Fortunately, the most serious metabolic consequences have seemed to selectively spare rats and man, a species difference that has made it feasible to perform the procedure with benefit to patients with glycogen-storage disease[33] and homozygous type-II hyperlipidæmia.[34] These applications accept a trade-off of distinctly suboptimal conditions of liver perfusion in return for metabolic improvements that almost certainly derive from the suboptimal conditions.[20 33 34] A similar weighing of gains and losses is necessary for the traditional indications for shunting operations in patients with œsophageal varices who retain hepatopetal portal flow. An obvious argument can be mounted for a Warren-type operation which preserves much of the residual splanchnic flow to the liver including that returning from the pancreas.[35]

Appreciation that there is a broader interplay between the liver and pancreas than the classical metabolic ones of Madison[36] and Felig and Wahren[37] should have ramifications in understanding the complications of diabetes mellitus, the pathogenesis of liver disease, and numerous other clinical problems as has been pointed out by others.[38 39]

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REFERENCES

1. Putnam, C. W., Porter, K. A., Starzl, T. E. *Ann. Surg.* in the press.
2. Mann, F. C. *J. Mt Sinai Hosp. N.Y.* 1944, **11**, 65.
3. Child, C. G., Barr, D., Holswade, G. R., Harrison, C. S. *Ann. Surg.* 1953, **138**, 600.
4. Fisher, B., Russ, C., Updegraff, H., Fisher, E. R. *Archs Surg., Chicago*, 1954, **69**, 263.
5. Fisher, B., Fisher, E. R., Lee, S. *Surgery Gynec. Obstet.* 1967, **125**, 1253.
6. Weinbren, K. F., Washington, S. L. A., Smith, C. Y. *Br. J. exp. Path.* 1975, **56**, 148.
7. Starzl, T. E., Francavilla, A., Halgrimson, C. G., Francavilla, F. R., Porter, K. A., Brown, T. H., Putnam, C. W. *Surgery Gynec. Obstet.* 1973, **137**, 179.
8. Starzl, T. E., Porter, K. A., Kashiwagi, N., Lee, I. Y., Russell, W. J. I., Putnam, C. W. *ibid.* 1975, **140**, 549.
9. Starzl, T. E., Porter, K. A., Kashiwagi, N., Putnam, C. W. *ibid.* 1975, **141**, 843.
10. Starzl, T. E., Porter, K. A., Putnam, C. W. *Lancet*, 1975, ii, 1241.
11. Felig, P., Gusberg, R., Hendler, R., Gump, F. E., Kinney, J. M. *Proc. Soc. exp. Biol. Med.* 1974, **147**, 88.
12. Loud, A. V. *J. Cell Biol.* 1968, **37**, 27.
13. Eck, N. V. K. *Voenno-med. Zh, S-Peterb.* 1877, **130**, 1. English translation: Child, C. G. *Surgery Gynec. Obstet.* 1953, **96**, 375.
14. Hahn, M., Massen, O., Nencki, M., Pawlow, J. *Arch. exp. Path. Pharmak.* 1893, **32**, 161.
15. Bollman, J. L. *Physiol. Rev.* 1961, **41**, 607.
16. Starzl, T. E., Marchioro, T. L., Rowlands, D. T., Kirkpatrick, C. H., Wilson, W. E. C., Rifkind, D., Waddell, W. R. *Ann. Surg.* 1964, **160**, 411.
17. Marchioro, T. L., Porter, K. A., Dickinson, T. C., Faris, T. D., Starzl, T. E. *Surgery Gynec. Obstet.* 1965, **121**, 17.
18. Marchioro, T. L., Porter, K. A., Brown, B. I., Faris, T. D., Hermann, T. J., Sudweeks, A., Starzl, T. E. *Surg. Forum*, 1965, **16**, 280.
19. Marchioro, T. L., Porter, K. A., Brown, B. I., Otte, J. B., Starzl, T. E. *Surgery, St Louis*, 1967, **61**, 723.
20. Starzl, T. E., Lee, I. Y., Porter, K. A., Putnam, C. W. *Surgery Gynec. Obstet.* 1975, **140**, 381.
21. Izzo, J. L., Bartlett, J. W., Roncone, A., Izzo, M. J., Bale, W. F. *J. biol. Chem.* 1967, **242**, 2343.
22. Blackard, W. G., Nelson, N. C. *Diabetes*, 1970, **19**, 302.
23. Field, J. B. *A. Rev. Med.* 1973, **24**, 309.
24. Rous, P., Larimore, L. D. *J. exp. Med., St Louis*, 1920, **31**, 609.
25. Meyer, W. H. Jr, Starzl, T. E. *Surgery, St Louis*, 1959, **45**, 760.
26. Tchobroutsky, G. *in* Diabetes (edited by W. J. Malaisse and J. Pirart); p. 667. Amsterdam, 1974.
27. Reaven, E. P., Peterson, D. T., Reaven, G. M. *J. clin. Invest.* 1973, **52**, 248.
28. Rubin, E., Gevurtz, N. R., Cohen, P., Tomita, F., Jacobson, J. B. *Proc. Soc. exp. Biol. Med.* 1965, **118**, 235.
29. Younger, L. R., King, J., Steiner, D. F. *Cancer Res.* 1966, **26**, 1408.
30. Holley, R. W. *Nature*, 1975, **258**, 487.
31. Leffert, H. *J. Cell Biol.* 1974, **62**, 792.
32. Bucher, N. L. R., Swatfield, M. N. *Proc. natn. Acad. Sci. U.S.A.* 1975, **72**, 1157.
33. Starzl, T. E., Putnam, C. W., Porter, K. A., Halgrimson, C. G., Corman, J., Brown, B. I., Gotlin, R. W., Rodgerson, D. O., Greene, H. L. *Ann. Surg.* 1973, **178**, 36.
34. Starzl, T. E., Chase, H. P., Putnam, C. W., Porter, K. A. *Lancet*, 1973, ii, 940.
35. Warren, W. D., Zeppa, R., Foman, J. J. *Ann. Surg.* 1967, **166**, 437.
36. Madison, L. L. *Archs intern. Med.* 1969, **123**, 284.
37. Felig, P., Wahren, J. *J. clin. Invest.* 1971, **50**, 1702.
38. Popper, H. *Gastroenterology*, 1974, **66**, 1227.
39. *Lancet*, 1975, ii, 1245.

"... it is essential to distinguish between arguments about whether Britain is devoting too much or too little of her national resources to particular social objectives and those about the methods of financing the provision of such services and benefits. For instance, it is at least conceivable that if public expenditure on the National Health Service were to be restrained to the point of affecting the quality of care provided, the result would be an increase in private spending on non-NHS provision: this might be desirable if the prime aim of policy is to limit the growth of public expenditure, but not if the aim is to hold back total spending on health care in order to free resources for industrial investment."—RUDOLF KLEIN, MARTIN BUXTON, QUENTIN OUTRAM, *Constraints and Choices*. Centre for Studies in Social Policy. £2.

ANTIBODY-DEPENDENT, CELL-MEDIATED CYTOTOXICITY AGAINST MELANOMA CELLS INDUCED BY PLASMAPHERESIS

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Summary Patients with disseminated melanoma were treated by repeated plasmapheresis using a continuous-flow blood-cell separator, as part of a study to investigate methods of removing factors from tissue fluids which block cell-mediated immunity. Using ^{51}Cr release cytotoxic assays, it was found that plasmapheresis resulted in removal of serum blocking activity. Post-plasmapheresis sera taken from several patients also increased cell-mediated cytotoxicity by induction of antibody-dependent cell-mediated killing. This effect may have resulted from removal or alteration of circulating immune complexes in the serum. It is not known whether cytotoxic activity induced in this way improves the patients' immune response against their tumours. However, the procedure is well tolerated and these preliminary in-vitro results indicate that this form of therapy could act as an adjunct to other forms of treatment of advanced melanoma.

Introduction

OBSERVATIONS in animal tumour models have suggested that tumours can grow in hosts immune to the tumour because of the production of factors which inhibit the cell-mediated cytotoxic response of the host.[1-5] A similar explanation may account for progressive tumour growth in man.[6-9] Studies in human malignant melanoma[10 11] have shown a correlation between the clinical growth of melanoma and the presence or absence of serum blocking activity against cell-mediated cytoxicity (C.M.C.).

We have attempted to determine whether removal of serum blocking activity of C.M.C. by plasmapheresis using a continuous-flow blood-cell separator would improve the immune response of melanoma patients against their tumours.

Patients and Methods

Patients

Four patients were studied, all having evidence of disseminated melanoma characterised by multiple subcutaneous nodules but no evidence of other visceral metastases as shown by clinical, radiological, or radionuclide scanning studies. Written consent was obtained from each patient.

Plasmapheresis

Plasmapheresis was carried out on an Aminco 'Celltrifuge' continuous-flow blood-cell separator with an average of 4 litres exchanged each time. Replacement fluids included physiological saline, Hartmann's solution, heat-treated stable plasma-protein solution (S.P.P.S.) (Commonwealth Serum Laboratories, Melbourne), human serum albumin, or 'Hæmaccel'