Oram *et al.*, 1964). The present evidence is such as to warrant observation of blood ureas in patients taking propranolol, though we have not found any rise of blood urea in normotensive patients.

Propranolol is a drug of considerable promise in angina pectoris and worthy of further evaluation. It remains to be determined whether or not such a high dosage is needed for maximum beneficial effect, though a previous trial (Srivastava *et al.*, 1964) at low dosage did not show a significant improvement in angina. The value of intermediate dosage is under investigation. Side-effects are not troublesome. Patients thought likely to develop heart failure should not be given propranolol, though if the subject has not been previously treated with diuretics and digitalis propranolol may be used cautiously after these drugs have been administered.

Summary

Propranolol was assessed in a double-blind trial in 14 patients with angina pectoris. The maximum tolerated dose, with an arbitrary upper limit of 100 mg. q.d.s., was used.

All 14 patients had fewer attacks of angina, consumed fewer glyceryl trinitrate tablets, and were subjectively improved on propranolol ($P = 0.00012$).

Side-effects were not troublesome with the use of propranolol.

ADDENDUM.—Since this paper was submitted a further trial, in which non-identical placebo tablets were used, has been published (Keelan, P. J. R. B., *Brit. med. J.*, 1965, **1**, 897). Unlike that of Srivastava *et al.* (1964), it did reach accepted levels of significance in favour of the active tablets. The dosage used was 30 mg. t.d.s. in all patients.

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Selective Lymphopenia by Use of Intralymphatic ^{198}Au and Splenectomy

Immunosuppressive Action on Rejection of Canine Renal Homografts

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Selective reduction of lymphocytes is a means of prolonging homograft survival (McGregor and Gowans, 1963). Intralymphatic irradiation of the lymphoid system with ^{131}I and ^{32}P produces selective lymphopenia (Tilak and Howard, 1964; A. Syrquin, personal communication). We report our preliminary experience with colloidal radioactive intralymphatic gold, which has greatly depleted the peripheral lymphocyte count and caused severe damage or destruction to the lymph nodes with little effect on the myeloid cells.

Method

Nineteen adult mongrel dogs ranging from 10 to 20 kg. were anaesthetized with intravenous barbiturate (thiopentone solution 0.5 g./100 ml. or pentobarbitone 30 mg./kg.).

One millilitre of patent blue violet was injected into the dorsum of the paw between the toes, and the lymphatics were then exposed and isolated 5–10 cm. proximal to the dye injection. Equal volumes of a sterile suspension of colloidal ^{198}Au stabilized with gelatin and 2.8% w/v sodium citrate at *p*H 6–7 with a particle-size of 200–300Å (20 millimicrons)[1] were then injected into the four extremities, using the cannulation method described by Kinmonth (1954). An identical fifth aliquot of colloidal ^{198}Au was injected direct into the mesenteric lymph-nodes at the time of splenectomy and renal transplantation. Kidneys were transplanted from unrelated dogs by vascular suture of the renal to the iliac vessels, the ureter being implanted into the bladder. With two exceptions (Dog No. 5, Table I, and No. 9, Table II) the dogs' own kidneys were removed at the same time. The animals were divided into two groups: Group I, colloidal ^{198}Au and splenectomy; Group II, colloidal ^{198}Au, splenectomy, and Imuran (azothioprine) daily from the time of renal transplantation. A third group of non-splenectomized control animals were treated with Imuran.

Group I (Six dogs).—Dose of colloidal ^{198}Au varied from 25 to 100 mc. In four dogs splenectomy, renal transplantation, and mesenteric node injections were performed seven days after the intralymphatic injections; in the remaining 10 animals all procedures were on the same day.

Group II (13 dogs).—Dose of colloidal ^{198}Au varied from 25 to 100 mc. In three dogs splenectomy, renal transplantation, and mesenteric node injection were performed seven days after the intralymphatic injections; in the remaining 10 animals all procedures were on the same day. Imuran was given from the day of renal transplantation at a dose varying from 10 to 2 mg./kg. daily by mouth.

Group III (10 dogs).—These were given Imuran 10 mg./kg. for two days, and then 5 mg./kg. daily by mouth.

The blood urea, white blood cell, and differential counts were performed three times a week. Post-mortem histology was obtained at intervals ranging from 2 to 66 days after the

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[1] Prepared at the Radiochemical Centre, Amersham.

intralymphatic injections. Lymph nodes were taken and examined separately from the popliteal, inguinal, iliac, mesenteric, mediastinal, axillary, and cervical groups. In addition, sections of the liver, renal transplant, intestines, lungs, and bone-marrow were examined.

The degree of destruction of the lymph nodes was graded from one to three plus: + = minimal destruction; + + = greater than 50% destruction; and + + + = complete destruction.

Renal homograft rejection was graded from one to three plus: + = minimal cellular infiltrate; + + = moderate cellular infiltrate and/or moderate vascular changes, more than 50% viable cortical tissue remaining; and + + + = severe cellular infiltrate and/or vascular changes, less than 50% viable cortical tissue remaining.

Results

In all gold-treated animals there was a selective lymphopenia (Tables I and II) which began on the second post-operative day and became progressively more severe until the seventh or eighth day and continued until the third to fourth week. The lymphopenia was much more pronounced than in the control dogs given Imuran alone (Table III). Fig 1 shows the typical response of an animal receiving 100 mc. and no Imuran. The total W.B.C. count was maintained, while there was a marked decrease in total lymphocyte count. Histological examination showed marked early depletion of the lymph-node population; thus by two weeks there was greater than 50% destruction of lymph nodes.

The dogs receiving 100 mc. did not show proportionately larger changes in lymphocyte count or lymph-node destruction

TABLE I.—*Data on Six Dogs (Group I) Treated with Intralymphatic ^{198}Au*

Dog	Dose ^{198}Au in mc.	Initial W.B.C. Count/c.mm.	Initial Lymphocyte Count/c.mm.	W.B.C. Count 14 Days After Intralymphatic Gold or at Death	Lymphocyte Count 14 Days After Intralymphatic Gold or at Death	Length of Survival in Days After Renal Transplant, and Causation of Death	Lymph-node Destruction	Homograft Reaction
1*	25	15,400	924	24,000	242	7 Pneumonia	+ +	0
2*	25	9,600	1,344	45,000	456	9 Rejection	+ +	+ + +
3*	25	18,000	1,820	36,000	360	9 Rejection	+	+ + + Cellular and haemorrhagic
4*	50	16,100	2,254	13,400	268	100 Alive	—	—
5	100	11,600	2,320	13,600	136	66 Intussusception	+ + to + + +	+ + Infarcted†
6	100	10,900	1,645	10,700	0	14 Uraemia	+ + to + + +	+ + + Non-cellular

* Splenectomy, renal transplantation, and mesenteric-node injection seven days after intralymphatic injections.
† Left with one remaining kidney in addition to transplant, which was removed at 14 days.

TABLE II.—*Data on 13 Dogs (Group II) Treated with Intralymphatic ^{198}Au and Imuran*

Dog	Dose ^{198}Au in mc.	Dose Imuran in mg./kg.	Initial W.B.C. Count/c.mm.	Initial Lymphocyte Count/c.mm.	W.B.C. Count 14 Days After Intralymphatic Gold or at Death	Lymphocyte Count 14 Days After Intralymphatic Gold or at Death	Length of Survival in Days after Renal Transplant and Causation of Death	Lymph-node Destruction	Homograft Reaction
1*	25	10 for 2 days then 5 daily	7,100	2,059	26,000	780	8 Rejection	+	+
2*	25	10 for 2 days then 5 daily	14,600	2,920	41,400	414	8 Intussusception	+ +	+
3*	25	10 for 2 days then 5 daily	14,600	2,920	21,000	1,000	24 Rejection	+ +	+
4	37·5	10 for 2 days then 5 daily	14,500	2,175	7,600	152	16 Pneumonia	+ + +	0
5	37·5	10 for 2 days then 5 daily	10,400	1,976	40,000	400	4 Intussusception	+ + to + + +	0
6	75	2–3	20,800	4,576	27,300	273	13 Rejection	+ + to + + +	+ + +
7	75	3–4	23,000	5,750	27,500	825	35 Rejection	+ + to + + +	+ + +
8	100	4	21,400	3,210	1,100	0	14 Pneumonia Marrow depression	+ + to + + +	0
9	100	3–4	29,800	4,470	9,200	280	19 Marrow depression	+ + to + + +	0
10	100	4	6,500	1,560	9,000	270	8 Intussusception	+ + to + + +	0
11	100	4	14,500	2,900	29,500	0	14 Ureter infarcted	+ + to + + +	0
12	100	4	15,800	—	—	—	2 Intraperitoneal haemorrhage	+ to + +	0
13	100	4	14,900	—	—	—	4 Peritonitis	+	0

* Splenectomy, renal transplantation, and mesenteric-node injection seven days after intralymphatic injections.

TABLE III.—*Data on 10 Dogs (Group III) Treated with Imuran*

Dog	Initial W.B.C. Count/c.mm.	Initial Lymphocyte Count/c.mm.	W.B.C. Count 14 Days after Commencing Imuran or at Death	Lymphocyte Count 14 Days after Commencing Imuran or at Death	Length of Survival in Days after Renal Transplant, and Causation of Death	Lymph-node Destruction	Homograft Reaction
1	10,200	1,428	6,300	1,449	18 Rejection	0	+ + +
2	10,700	1,712	9,000	2,070	27 Rejection	0	+ + +
3	9,100	1,547	10,000	2,500	29 Rejection	0	+ + +
4	11,300	226	11,200	448	12 Pneumonia. Rejection	0	0
5	20,300	1,624	21,100	1,680	9 Intussusception	0	+
6	15,000	1,500	5,900	531	20 Pneumonia. Rejection	0	+
7	8,500	408	7,000	420	24 Pneumonia.	+	0
8	10,850	3,247	12,800	2,688	21 Pleural effusion	+	+
9	16,000	2,968	26,700	1,335	13 Rejection	0	+ +
10	16,000	1,482	10,500	1,260	13 Intussusception	0	+

All dogs treated with Imuran 10 mg./kg. for two days, and then 5 mg./kg. daily.

than those receiving 37.5 mc. However, lymphopenia was less severe in those given 25 mc.

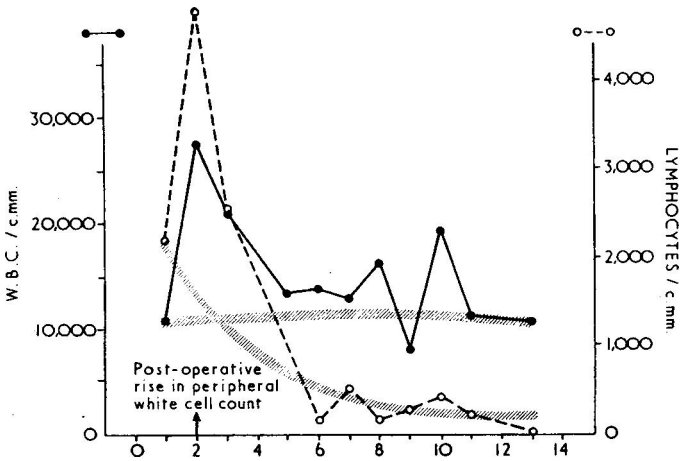

FIG. 1.—Total white blood cell and lymphocyte counts on Dog 6 (Table I) treated with 100 mc. ^{198}Au and splenectomy. The hatched lines show the trends of the counts without the post-operative leucocytosis. There is marked selective lymphopenia.

In Groups I and II the early changes in the axillary, iliac, aortic, and mesenteric nodes showed a decreased population followed in about one week by nearly complete replacement of the node with haemorrhage and disappearance of the normal lymphocyte population (Fig. 2). After about 30 days the nodes were surrounded by fibrous tissue with only a shell of amorphous material representing the lymph node. The cervical and mediastinal nodes showed some depletion with no fibrosis. The dogs receiving Imuran alone had only minor changes in the lymph nodes, in marked contrast to the ^{198}Au-treated animals.

In untreated dogs transplant function usually ceases five to eight days after renal transplantation, with microscopical changes in the ++ to +++ range (Dempster, 1953; Simonsen, *et al.*, 1953). In Group I rejection was most rapid and severe in the animals treated with 25 mc. ^{198}Au; while the dog given 50 mc. has good function at 100 days, the dog given 100 mc. died from rejection at 14 days. In animals dying with no peripheral lymphocytes, rejection when present consisted mainly of vasculitis and haemorrhage. In Group II three transplants at 14–19 days had a normal appearance with no cellular infiltration or vasculitis (Fig. 3). In Group III nine transplants between 10 and 29 days showed rejection; one at 24 days was microscopically normal.

FIG. 2.—Photomicrograph of popliteal node following injection of 20 mc. ^{198}Au into afferent lymphatics. Shows gross destruction with no remaining lymphoid cells. (Haematoxylin and eosin. ×160.)

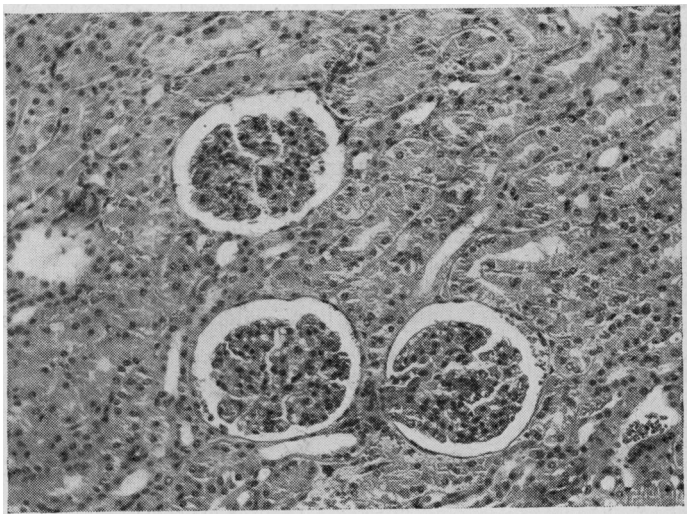

FIG. 3.—Photomicrograph of renal transplant at 16 days from dog treated with 100 mc. of intralymphatic ^{198}Au, splenectomy, and Imuran. Shows normal appearance with no cellular infiltrate. Dog 8 (Table II). (Haematoxylin and eosin. ×160.)

Discussion

Current methods of immunosuppression utilized in clinical renal transplants are unsatisfactory owing to their poor therapeutic index. It is not uncommon for rejection to proceed relentlessly in spite of lethal doses of anti-metabolite drugs and steroids. There is no doubt that lymphoid cells can bring about the destruction of transplanted tissue, and there is no direct evidence that cells other than those in the reticulo-endothelial system are capable of similar immune action.

One of the main difficulties in the use of anti-metabolite drugs is that they impair function of all actively dividing cells. Thus damage to the erythroblastic, and, more important, the myeloid elements of the bone-marrow may prevent adequate immunosuppressive doses being used.

Intralymphatic ^{198}Au has been used by Jantet (1962) for treatment of lymph nodes in malignant melanoma. His technique produces gross damage to the draining nodes in human patients. Our results show that this isotope, injected intralymphatically in dogs, will produce similar selective lymphopenia to that described by Tilak and Howard (1964) and by Syrquin (personal communication), with ^{131}I and ^{32}P respectively. Injecting the four limbs and the mesenteric nodes directly, together with a surgical splenectomy, must remove or destroy a considerable proportion of lymphoid tissues of the body. It was of interest that not only were the circulating lymphocyte counts greatly reduced, but the lymphoid-node histology in glands not directly draining the injection sites—namely, the cervical and mediastinal nodes—were also moderately depleted. It is possible that particles of radioactive colloidal gold were carried to these other lymphopoietic sites by reticulo-endothelial cells from the draining nodes, although we have no direct evidence of this. Splenectomy alone does not prolong the survival of renal transplants in dogs, nor does it improve the immunosuppressive action of Imuran (Starzl *et al.*, 1964).

In our experiments radioactivity was present in the blood immediately after injection of colloidal gold. Gold without

Imuran was not sufficient to cause neutropenia. However, there may have been additive marrow toxicity in the dogs given both Imuran and gold.

These preliminary results show that 37 mc. or more of intralymphatic radioactive gold prolongs renal homograft survival approximately twice that of normal. The results of ^{198}Au in combination with Imuran suggests added immunosuppression, but the numbers in each experimental group are insufficient for statistical analysis. Modification of the initial protocols with regard to dosage and timing of radioactive gold in relation to the renal transplantation is currently being undertaken in an attempt to improve the therapeutic effect.

Summary

Intralymphatic irradiation with colloidal ^{198}Au combined with splenectomy and direct injection of the isotope into the mesenteric lymph nodes produces a marked selective lymphopenia in the dog, which lasts for three to five weeks. The rejection of homologous renal transplants in these animals is delayed. Combination of intralymphatic irradiation plus Imuran probably results in additive toxicity but also additive immunosuppression. Severe lymph-node destruction is produced by the irradiation.

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One of us (J. R. W.) is a post-doctoral fellow, 1-F2-HE-24, 158-01, National Heart Institute, U.S. Public Health Service.

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Medical Memoranda

Pregnancy After Primary Irradiation for Carcinoma of Cervix

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A successful pregnancy after radiation therapy for carcinoma of the cervix is described.

Case Report

A 31-year-old woman was admitted to the Government Hospital for Women and Children, Madras, in February 1957. She gave a history of irregular vaginal bleeding of about 18 months' duration since the delivery of her last child. She had had five full-term natural deliveries, and two abortions at the fourth month. She was well-nourished, and systemic examinations were negative except for a stage I carcinoma of the cervix, which was confirmed histologically. She was treated with radium 7,000 r to point A, which was followed in April 1957 by an extraperitoneal pelvic lymphadenectomy. The lymph nodes showed no metastases.

She was discharged in April 1957 with a healed lesion, and was asked to report for a follow-up.

The patient was seen on two occasions, once in May 1961 and then in January 1964 with a history of vaginal bleeding of seven and four days' duration respectively. On both occasions the cervix could not be felt, the uterus was normal, and the fornices were mobile. Rectal examination on both occasions also showed the parametria to be free. Vaginal smears taken at both follow-ups showed no malignant cells, and a biopsy taken in 1961 of a nodule on the vaginal vault showed no evidence of malignancy.

On 24 September 1964—that is, about nine months after the last vaginal spotting of blood—she was admitted in labour with a full-term pregnancy. There was no clinical evidence of a recurrence of the cervical lesion. As on previous occasions the cervix could not be felt, a caesarean section was performed, and a live girl weighing about 8 lb. (3.6 kg.) was delivered. The ovaries were macroscopically normal. The baby had no congenital malformations. Both mother and baby are at present doing well.

Comment

With the standard radiation programme of combined radium and *x*-ray therapy that is currently employed in treating

Summary of Reported Cases

Interval to Subsequent Pregnancy	Mode of Delivery	Condition of: Child	Condition of: Mother	Year Therapy Given	Author	Age of Patient	Radiation Dosage	Status of Patient When Treated
5 years	Vaginal	Normal at 8 yr.	Well at 5 yr. Had three abortions before successful pregnancy	1913	Döderlein (1922; 1928)	31	2,510 mc h	? 6 weeks post abortion
2 ,,	Caes. section	Normal at 3 yr.	Well at 3 yr.	1920	Siredey (1923)	24	2,100 mc h	10 weeks pregnant
8 months	Vaginal	Normal	Died from post-partum haemorrhage	1922—1924	Ikeda (1927)	—	About 10,000 mc h	Second half of pregnancy
1½ years	,,	,,	,, ,, ,,	,,	,,	—	,, ,,	,, ,, ,,
2 ,,	,,	,,	,, ,, ,,	,,	,,	—	,, ,,	,, ,, ,,
2 ,,	,,	,,	Well at 5 yr.	1924	Philipp (1932)	35	7,000 mc h	4 months pregnant
1 year	,,	Normal at 3 yr.	Well at 4 yr.		Wickham and Touflet (cited)	—	39 mc d	—
5 years	Caes. section	2 children both normal at 7 and 2 yr.	Well at 12½ yr.	1950	Whelton and McSweeney (1964)	21	5,000 mc h	6 weeks post-partum
1 ,,	,,	Normal	Well at 2 yr.	1959	Ivey (1963)	26	8,000 r to point A	Not pregnant; large exophytic tumour
7 ,,	,,	,,	Well at 4 months	1964	Francis and Stevens	38	7,000 r to point A	1½ years post-partum