The Radiomimetic Nature of Bracken Toxin

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SUMMARY

Young, actively growing bracken contains a toxin(s) which reproduces many of the effects of radiation. It produces intestinal adenocarcinomas in rats and Japanese quail, pulmonary adenomas in mice, and hematuria and bladder tumors in guinea pigs. Age dependence has been established for rats. An active fraction containing both the acute cattle factor and the carcinogen is mutagenic (drosophila and mice), and this test is being used as a small scale assay for the final stages of isolation of the activity. The gastrointestinal and bone marrow deaths have been reproduced in calves and lambs. The calf model has been used for an intensive study of the early syndrome. Humoral and cellular changes (including mast cells) are reported.

INTRODUCTION

The aim of this paper is to take a perspective view of the work done on the radiomimetic nature of the bracken toxin during the last seven years, in collaboration with a group of postgraduate research students. Although the work will cover a wide and apparently diverse field, it is hoped that there will be sufficient evidence to show the interdependence of the various sectors and the value of each towards a fuller understanding of the problem as a whole.

The death of cattle from acute bracken poisoning has been recognized for many years, and the typical hemorrhagic syndrome described. Animals succumb after a period of roughly three to ten weeks, often with mucoidal nasal and anal hemorrhage (which can be massive), and severe anorexia and pyrexia. The latter is not alleviated by wide-span antibiotics used to combat any possible bacteremia. Postmortem reveals predominantly widespread hemorrhages of all sizes, the gut being particularly badly affected usually with ulcers, and often to a lesser extent the heart.

Schofield (54), Evans et al. (12), and Naftalin and Cushnie (44) recorded the hematologic changes, namely, leucopenia and agranulocytosis, thrombocytopenia, increased capillary fragility, and impaired clot formation. Sternal bone marrow punctures clearly showed the suspected progressive hemopoietic damage resulting in aplastic anemia (18-20, 45).

We drew an analogy between the clinical picture of our bracken calves and the bone marrow lesion produced by X-radiation and by radiomimetic chemicals (15). This was amply confirmed by the description of the results of whole-body γ-radiation of calves (55) and in five-year-old Hereford cattle (5, 6). Most deaths occurred around the three-week period after a steep fall in leukocytes and thrombocytes due to acute hypoplasia of the bone marrow. Mucous- and blood-stained feces were notable and terminally there was anorexia and severe pyrexia. Autopsy revealed widespread hemorrhages, massive in the intestines (which were also ulcerated), and markedly in the heart.

In the subject under review the general interest in radiomimetic chemicals is enhanced by the fact that this one is naturally occurring and produced by a plant which is not only old, but extraordinarily widespread on the earth's land surface. A comparative review of the biologic properties of many of these radiomimetic compounds is given by Elson (11); Boyland (4) lists twenty-five biologic effects of radiation within which they could be categorized. In this paper it is proposed to follow the plan outlined in Table 1 which distinguishes four main divisions in the radiation syndrome based on the time-dose variations. Although not in the chronologic order in which the work proceeded, Section 4 will be dealt with first, followed by Section 2, and finally by Section 3. In this last section the calf is used as a model to study the physiologic and pathologic changes accompanying the delayed bone marrow syndrome. The relevance of Section 3 to the clearer understanding of the initial stages of carcinogenesis will be apparent.

LONG-TERM EFFECTS (SECTION 4)

Rosenberger and Heeschen (51) had already reported that changes of a polypous-tumorous nature in the bladder mucosa accompanied hematuria in 5 cattle fed bracken for long periods. Georgiev et al. (23) working with an extract of urine of cattle fed hay from hematuria districts, produced hemangioma-like lesions when it was introduced into the urinary bladders of dogs, and papilloma type excrescences on mouse skin. Pamukcu (46) had made an epidemiologic survey in Turkish cattle of the condition known as chronic enzootic bovine hematuria and the associated bladder tumors. The etiology was not known but it appeared consistently where bracken flourished and had a worldwide distribution causing heavy losses in some areas. The possibility of a viral agent was also suggested and the transmissibility of the tumors tested. Suspensions of naturally occurring bladder tumors from Turkish cattle produced fibropapilloma in the skin and vaginal mucosa of test calves. In 1964 we decided to test directly the possible carcinogenic activity of bracken with sufficient numbers of assay animals on which to base a firm conclusion (14, 41).

June bracken carefully dried for 1 hour in a tumbler drier at 50°C, followed by 48 hours in an oven at 40°C, was milled

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Table 1

<table>
<thead>
<tr>
<th>Hours</th>
<th>Days</th>
<th>Weeks</th>
<th>Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CENTRAL NERVOUS DEATH</td>
<td>2. GASTROINTESTINAL DEATH</td>
<td>3. BONE MARROW DEATH</td>
<td>4. CANCER DEATH</td>
</tr>
<tr>
<td>Convulsions</td>
<td>Destruction of GI mucosa</td>
<td>Delayed bone marrow aplasia</td>
<td>a) Local, e.g., bone, skin</td>
</tr>
<tr>
<td>Immediate death</td>
<td>Bone marrow aplasia</td>
<td>Hemoerhages</td>
<td>b) Whole body, e.g., leukemia, gastrointestinal, lung, mammary</td>
</tr>
<tr>
<td></td>
<td>Electrolyte loss</td>
<td>Delayed gastrointestinal syndrome</td>
<td>(Species and strain dependent)</td>
</tr>
<tr>
<td></td>
<td>Systemic toxemia</td>
<td>Histamine and heparin release</td>
<td>INDUCTION OF MUTATIONS.</td>
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Decreasing Dosage

The radiation syndrome.

and made into pellets with the powdered normal rat diet at the rate of 33% bracken by weight. This was fed ad lib to 7-week-old Lister, noninbred rats, twenty of each sex, for a period of 64 days. Parenteral B1 was given three times during the course of bracken feeding to counteract the thiaminase present in bracken (21, 22), and 40 control animals received the same treatment. Between 7 months to 1 year from the start of feeding all the bracken rats succumbed to multiple adenocarcinoma of the intestine, predominantly in the ileal region, compared with 0% in the controls. All stages of the tumors are to be found up to the most malignant forms, intussusceptions are frequent, and often very large diverticuli are formed and encroach on the abdominal space. The first signs of the cancerization are intermittent scouring which becomes progressive (Figs. 1 and 2).

Subsequent experiments confirmed these findings (16, 60) and, in addition, it was found that if the rats were older at the start of feeding (13 to 18 months) they failed to develop ileal tumors, demonstrating that age susceptibility is also a factor in bracken-induced cancer as well as with radiation. The possibility that a nonspecific dietary irritation was the etiologic factor was eliminated by showing that the solid residue of bracken after extraction is not carcinogenic.

Rat intestinal tumors are not frequently encountered. Schoental and Bensted (53) and Schoental (52) report their induction by radiation and polycyclic aromatic hydrocarbons and Laqueur (39) produced them by feeding cycasin. The latter is of interest since it is another naturally occurring carcinogen (from a tree fern), itself inactive but which is broken down to the aglycone by gut bacteria. The aglycone is a dialkylaminoalkylamine and so belongs to a group of carcinogens which is known to produce tumors in different sites in the body and is thought to have a wide range of action in different species (8, 40).

We have found just such a species variation to obtain with bracken. Noninbred Swiss white mice (females aged 6 weeks) when fed on the bracken pellets for 5 weeks still showed nothing after about 1 year but had, in fact, been developing pulmonary adenomas, and none survived beyond 19 months. Again the incidence in all our bracken mice which have been autopsied is 100%. The average number of peripheral lung adenomas per mouse in 26 mice is 16, with a minimum number of 5 and a maximum number of 30. For 46 control mice the average is 0.57 per mouse, most having none, and only one mouse having a maximum of three. No mouse, bracken fed or control, has produced an intestinal tumor although careful search has been made by lavage and filling with fixative. We are now using mice as the assay animal of choice since it became apparent that the peripheral adenoma count is semiquantitative, and that an answer can be obtained after only 6 months. Many mice can be used per group, and there are already, after this time period, enough tumors present to give statistically accurate results (60). R. Jones and the writer are at present attempting to reduce the time still further by bracken feeding the mothers throughout pregnancy and lactation in the hopes of inducing neonatal carcinogenesis in the offspring, as described by Shimkin (56) and Smith and Rous (57). At the same time, the effects of skin-painting mice with an active fraction are being investigated, using the resting period of hair growth described by Berenblum et al. (2). If bracken toxin has to be activated enzymically in the same way as cycasin, a negative result might be anticipated for the skin test of the native material.

Japanese quail (Coturnix coturnix japonica) have been fed with an active hot ethanol extract of dried June bracken mixed with their normal diet for the first 5 months after hatching. Fatalities occurred from 5 months of age onward and are caused by highly malignant adenocarcinoma predomi-
nantly in the two caecae, which become increasingly hemorrhagic; and also in the colon, with a smaller number still in the distal ileum. The remaining animals were sacrificed when 10 months old and from a total of 34 animals (both sexes) the incidence of adenocarcinoma had reached 79.5%. A group of 34 control quail failed to produce any tumors (17) (Fig. 3).

A group of 13 guinea pigs (6 weeks old) were given a supplement of fresh bracken fronds ad lib to their normal diet for a period of 77 days. One of these animals succumbed to adenocarcinoma of the jejunum after 23 months. Between the 17th and 28th month after the start of feeding, 9 out of 13 guinea pigs had developed chronic, intermittent hemorrhages from the urinary bladder. The animals were mostly killed when in very poor condition and the experiment terminated after 30 months. Autopsy revealed the following pathologic changes occurring in the bladders: epithelial hyperplasia occurred in all of the animals, this advanced through papillomatous changes to papillary carcinoma (occasionally adenocarcinoma); frequent infiltrating carcinoma and transitional-cell carcinoma, sometimes with glandular or squamous metaplasia; Brunn's nests were present in nearly every section; and chronic inflammatory infiltration was seen with submucosal hemorrhages and sometimes fibrosis. Three bladders contained stones and two animals had hydronephrosis. One animal had a gelatinous appearance in the region and another in the pelvic region. Six control guinea pigs showed no changes and the bladders were normal. White urethral plugs were found in both bracken and control guinea pigs.

Pamukcu et al. (47) have produced the typical bovine enzootic hematuria syndrome in 10 out of 18 cattle fed varying amounts of bracken over a mean period of 550 days. They developed neoplasms of the bladder, 3 of which were transitional-cell carcinoma, sometimes with glandular or squamous metaplasia; Brunn's nests were present in nearly every section; and chronic inflammatory infiltration was seen with submucosal hemorrhages and sometimes fibrosis. Three bladders contained stones and two animals had hydronephrosis. One animal had a gelatinous appearance in the region and another in the pelvic region. Six control guinea pigs showed no changes and the bladders were normal. White urethral plugs were found in both bracken and control guinea pigs.

In the case of K this could be related to the systemic cell damage after a few days to about a fortnight. There is pyrexia, weight loss, destruction and denudation of the gastrointestinal mucosa, characterized by cessation of the mitotic activity of the crypt cells, and severe electrolyte loss which may be associated with diarrhea (34, 35).

GASTROINTESTINAL DEATH (SECTION 2)

Generally speaking this syndrome is produced by higher levels of radiation and precedes the bone marrow death, although damage to the blood-forming organs is already underway. The condition is fatal with death occurring from a few days to about a fortnight. There is pyrexia, weight loss, destruction and denudation of the gastrointestinal mucosa, characterized by cessation of the mitotic activity of the crypt cells, and severe electrolyte loss which may be associated with diarrhea (34, 35).
villi, very few mitoses, and no goblet cells. On the other hand, plasma and lymphocytic infiltrations were prominent, and the organs as a whole were distended and filled with fluid.

It was clear that neither animal had received sufficient toxin to precipitate the true GI death, and complicating features may be a slow rate of bile Na secretion in calves, coupled with an ability to absorb Na across a considerable concentration gradient in the lower part of the intestine. In order to step up the toxic dose/kg body weight, it was clear we had to administer a concentrated extract by drench to a young animal. Although sheep are recognized as being more resistant to bracken than cattle (particularly when adult), they can develop the bone marrow syndrome if allowance is made for the facts that they do not readily eat bracken and the individual resistance is very variable (33, 42, 43, 48, 60).

By drenching an 8-week-old Welsh mountain lamb with an extract containing the equivalent of 600 gm dried bracken per day, Widdop (60) succeeded in producing the classic GI syndrome in 12 days. At that time the animal was killed, being almost moribund, in considerable pain, and having passed a large amount of blood in the feces. Chart 1 shows the catastrophic fall in leukocytes and platelets, the resistance of erythrocytes, and possible hemoconcentration. The lymphocytes fall immediately and the neutrophils equally sharply after an initial increase. Autopsy revealed the peritoneal cavity contained a lot of fluid (another sign of hemoconcentration), fewer hemorrhages than in the bone marrow death, impaction of the rumen, and severe damage to the whole length of the gut, which was suffused with blood. The lining was being sloughed off into the lumen and the greatest damage occurred in the small intestine. Fig. 5 shows the condition to be identical with that produced by radiation described by Bloom (3). The villous and crypt epithelium is destroyed and disintegrating, with cystlike dilatations at the base of the crypts, often containing debris, absence of goblet cells, enlargement of some of the epithelial cells and nuclei, clumping of chromatin and pyknotic appearance to many of the mitotic figures, edema and hemorrhage into the submucosa, with cellular infiltration. It is thought that the electrolyte balance experiment, if applied to such an animal, would give a positive result.

THE BONE MARROW DEATH (SECTION 3)

A characteristic humoral change produced by radiation is the increase in concentration of the low density β lipoproteins and the decrease in the dense α lipoproteins (25, 30). This has been confirmed in bracken calves by Tong (59), and she has further demonstrated a definite increase in nonspecific lipolytic activity and a suggested increase in “clearing factor.” The association of the latter with the endogenous release of heparin (mentioned below), makes the calf an interesting model for the study of this interconnected function which has been described by Hahn (27), Graham et al. (26), Robinson and French (50), and Kern et al. (37). Permanent damage to the wall of the blood vessel can be caused by radiation and it is well known that altered fat metabolism, endothelial damage, and increased permeability of ground substances are all involved. Work on this aspect of bracken toxicology is currently being developed by Leach.

Another change in blood protein is the large terminal increase in fibrinogen (9, 13, 28, 31). Howell and Evans (32) further describe qualitative as well as quantitative changes in seromucoids and fibrinogen, the latter being due to molecular aggregation. Heparinoid substances are known to form reversible polymers with fibrinogen, and Jacobson et al. (36) record increased amounts of heparin in the blood of irradiated rabbits. We did indeed find marked, discontinuous increases in circulating heparin in terminal bracken calves. This was confirmed by Mason (41), together with a similar pattern of blood histamine.

All these changes are characteristic of an acute, systemic inflammatory condition which may be an integral part of the syndrome. Bacteremia rarely occurs in the experimental animal receiving wide-span antibiotics, but the possible importance of absorption of bacterial endotoxin through the damaged gut cannot be ruled out. The generally held opinion that radiation suppresses immunologic reactions would seem to run counter to the idea of an inflammatory event, but there are not a few references which suggest that the situation is more complex and that alteration of native molecules by ionization could induce an adverse state of autosensitization (38, 49). This could be the case with the bracken toxin since chemical alteration or combination of the hapten type is known to occur with some drugs (29). Enhanced antibody formation could be invoked (10) and/or a cell-bound immunologic system such as the homograft type.

In order to tackle this problem, we have taken biopsy...
Chart 2. Calf fed 100 gm dried bracken/day for 40 days followed by 1000 gm/day till death. Blood counts, tissue mast cells, and circulating heparin and histamine.
samples of the subcutaneous connective tissue concurrently
with the blood samples from calves receiving bracken. Experience
has shown that the changes are marked and clear, but
often happen in such rapid succession that daily sampling in
critical periods is advisable. One of the most striking features
found is the very large discontinuous increases in mast cell
numbers which occur terminally, or at times of low circulation
of leukocytes and platelets, and are generally found to precede
the increases in circulating heparin and histamine. The rate of
turnover of the mast cells is astonishing, and poses quite a
problem as to their origin (7, 24).

Accompanying the mast cell increase are extravascular
invasions of all intensities including lymphocytes, plasma cells,
monocytic types, and eosinophils. Extravascular neutrophils
are rarely seen. It is important to realize that the differential
blood count gives no indication of the numbers of cells in the
tissues, e.g., on a day when there are no circulating eosinophils
the tissues can be swarming with them. At such times of
activity, changes in the ground substance and endothelial cells
can also be seen.

In two cases where the calf had received a low sensitizing
dose of bracken for a period before the normal dose, the
biopsies gave every appearance of an acute delayed hypersensi-
tivity reaction, and giant cells containing large numbers of
peripheral nuclei occurred at the times of mast cell peaks.
There have also been calves in which mitotic activity could be
recorded. Chart 2 gives the parameters for a calf which
illustrated both of the last two observations. The mitotic
record would seem to be most valuable since it shows that very
marked systemic changes occur after only a few days on
bracken and long before the major pathologic events yet, they
may well be an important factor in the initiation of carcino-
genesis (31, 41, 59, 60).

One of the most encouraging signs that inflammatory events
are playing a major role is the result of parenteral therapy with
a synthetic hydrocorticosteroid (Betsolan, Glaxo, Ltd.). These
drugs are known to suppress lymphocytes, plasma cells, mast
cells, and eosinophils, but conversely promote neutrophilia
and monocyte production. They stabilize membranes and are
used extensively in a wide variety of inflammatory disorders.
Widdop (60) records that 3 out of 5 seriously affected calves
recovered with a markedly rapid myelopoiesis. The dangerous
side effects of the steroid on the already damaged intestines
accounted for the other two, but they yielded the valuable
information that the hemorrhages were regressing rapidly and
the bone marrow showing signs of recovery.

The many facets of information gained from this calf work,
together with the positive results with betamethasone, seem to
indicate that membrane damage and enzyme release (1) may
play a vital role in the course of radiation-type disorders and
future work with bracken will be oriented in this direction.

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Fig. 1. Ileum of bracken-fed rat showing multiple adenocarcinomas and a very large diverticulum (9 months).
Fig. 2. Transverse section. Adenocarcinoma in the ileum of bracken-fed rat showing multiple metastases through the wall × 20.

Fig. 3. Transverse section. Large adenocarcinoma distending one cecum and both ceca hemorrhagic, in quail fed bracken extract (6 months). × 20.
Fig. 4. Transverse section. Wall of urinary bladder of bracken-fed guinea pig. Infiltrating carcinoma in muscle layers (23 months). × 40.

Fig. 5. Longitudinal section. Ileum of Lamb A showing the destruction of the mucosa, with cystlike dilatations of bases of the crypts and submucosal hemorrhage. Mallory × 40.
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