A MEASUREMENT OF METANEPHRINE AND NORMETA-
EPHINE BY RADIOIMMUNOASSAY. H.Tashiro,
A.Takeyasu and T.Ishigami. Kitasato
Biochemical Laboratories (Bristol-Myers KK).
Sagamihara.

A measurement of metanephrine and nor-
metanephrine has been identified in central and
peripheral neural tissues and others.

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RADIOIMMUNOASSAY of CGRP. K.Kanao, S.Ishihara,
M.Honda, M.Usami, H.Ochi and H.Mori.
Sumitomo Hospital and Osaka City University.
Osaka.

Calcitonin Gene-Related Peptide (CGRP) is a
discovered peptide of 37 amino acids, and has been identified in central and
peripheral neural tissues and others.

We reported fundamental evaluation on CGR
P radioimmunoassay. The CGRP assay was
conducted by adding to 100 µL of either
standard (0–5,000 pg/mL) or sample, 100 µL
of 150 tube/vial diluted rabbit antiserum
against hCGRP (Amerham), 500 µL of 50 mM
sodium phosphate buffer at pH 7.4 contain-
ing 0.2% BSA, 10 mM EDTA, 0.1% NaN3.

The mixture was incubated overnight at
4°C, and then 100 µL of I-125 hCGRP (less
than 107 Bq/mL, Amerham) were added in
each test tubes. The mixtures were in-
culated at 4°C for three days. Antibody
bound CGRP was then precipitated by adding
of 300 µL of a second antibody. Sensitivity
was 40 pg/mL and normal value (20–40
years) was 80–280 pg/mL (n=15). There was
no interference with the assay from Calci-
tonin, Osteocalcin, and Somatomedin-C.

CGRP in three patients with MTC
(Medullary thyroid carcinoma) was 260,570
and 15,000 pg/mL respectively. Gel perma-
nence chromatography of this MTC sample,
shows 15,000 pg/mL, extractable CGRP-like
immunoreactivity revealed two distinct
immunoreactive peaks.

RADIORECEPTOR BINDING OF CHOLECYSTOKININ
TO GASTRIC CHIEF CELLS. M.Noguchi, H.Adachi,
S.Sato, T.Honda, S.Ohishi, E.Aoki and
K.Torizuka*. Kyoto University School of
Medicine, Kyoto and Fukui Medical School*
Fukui.

Cholecystokinin (CCK) stimulates pepsi-
nogen secretion from gastric chief cells of
guinea pig. We examined radio receptor
binding of CCK to isolated chief cells from
guinea pig stomach. Gastric chief cells
were prepared from gastric mucosa by enzyme
digestion using collagenase and subsequent
centrifugation with percoll density gra-
dient. Natural CCK = 33 was iodinated by
the method of Bolton-Hunter. Binding of I-125
labeled CCK to chief cells was saturable,
reversible and dependent on temperature.
Unlabeled CCK, but not VIP, inhibited the
binding. Hoftree plot (modified Scatchard
plot) obtained from dose-response inhibi-
tion curve of CCK-8 for the binding, showed
that CCK receptor on chief cell possessed
one high-affinity binding site (Kd value:
0.4 nM). When compared the action of CCK
for the binding with that for pepsi-
nogen secretion from chief cells, both response-
curves consisted with each other, indicating
that the receptor binding of CCK to chief
cells is physiological. In addition, IgG
purified from control sera using Protein A
affinity to affect the binding
at the concentration below 50 µg/mL.
These results suggest that the receptor
binding assay of CCK to chief cells is use-
ful to detect anti-chief cell antibody in
serum of patients with pernicious anemia.

RADIOIMMUNOASSAY for 11-DEOXYCORTICOSTERONE
USING NEW HAPTEN. M.Hachinoe, T.Tanaka,
H.Harada and A.Kubodera. Faculty of
Pharmaceutical Sciences, Science University
of Tokyo, Tokyo.

Several types of hapten have been made
to prepare antisera for the radioimmuno-
assay (RIA) of 11-deoxycorticosterone (DOC).
The antisera so far obtained, however, are
not yet satisfactory with the specificity.
Therefore we prepared the DOC-BSA conjugate
having the bridge at the C-4 position in
the C-1-ketosteroid molecule which was
used to yield the specific antisera for
DOC, and attempted to establish the RIA for
DOC.

DOC was transformed into the 4-(2-
carboxyethylthio) ether as a hapten, and
the male guinea pigs were immunized with
the hapten-BSA conjugate. The affinity,
sensitivity and specificity of the
resulting antisera were assessed.

The antisera exhibited the high affinity
for DOC with an affinity constant (K of:
1.27 x 109 M-1). The RIA method is capable
determining DOC in the range of 20–1000 pg
by using H-3-DOC (a.a. 42 Ci/mmol). The
antisera could well recognize the 11
A and the functional groups on the C-17 side
chains, and the specificity of them was
improved as compared with the conventional
antibodies. Furthermore, the antisera were
purified by eliminating cross-reactive
antibodies by affinity chromatography, and
the specificity of the purified antisera
was assessed.